SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL N-MANNICH BASES OF BENZIMIDAZOLE DERIVATIVES

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R. Medical University,

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In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

Submitted by

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Reg. No: 26106037

Under the Guidance of

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Associate Professor

(Department of Pharmaceutical Chemistry)

ADHIPARASAKTHI COLLEGE OF PHARMACY

(Accredited by "NAAC" with CGPA of 2.74 on a Four point Scale at "B" Grade)

MELMARUVATHUR - 603 319

MAY - 2012

CERTIFICATE

This is to certify that the research work entitled **"SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL N-MANNICH BASES OF BENZIMIDAZOLE DERIVATIVES"** submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment for the award of the Degree of Master of Pharmacy (Pharmaceutical Chemistry) was carried out by **SREERAMA RAJASEKHAR (Reg. No: 26106037)** in the Department of Pharmaceutical Chemistry under my direct Guidance and Supervision during the academic year 2011-2012.

Place: Melmaruvathur

Date:

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Melmaruvathur – 603 319.

CERTIFICATE

This is to certify that the dissertation entitled **"SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL N-MANNICH BASES OF BENZIMIDAZOLE DERIVATIVES"** is the bonafide research work carried out by **SREERAMA RAJASEKHAR (Reg. No: 26106037)** in the Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R Medical University under the guidance of Mr. **M. SUGUMARAN,** M. Pharm., (Ph.D.), Associate Professor, Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, during the academic year 2011-2012.

Place: Melmaruvathur

Date:

Prof. (Dr.). **T. VETRICHELVAN,** M. Pharm., Ph.D.,
Principal,
Adhiparasakthi College of Pharmacy,
Melmaruvathur – 603 319.

Dedicated to

My

Parents and friends

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Finally yet importantly, I gratefully forward my affectionate thanks to my family members, friends, which has sustained me a lot in the successful completion of my project work**.**

SREERAMA RAJASEKHAR

CONTENTS

LIST OF ABBREVIATIONS

IR	-	Infra red
%	-	Percentage
° C	-	Degree Centigrade
µg	-	Microgram
ATCC	-	American Type Culture Collection
DHFR	-	Dihydrofolate reductase
DMF	-	Dimethyl formamide
FT-IR	-	Fourier Transform Infra red spectroscopy
g	-	Gram
h	-	Hour
HIV	-	Human Immunodeficiency Virus
HOMO	-	Highest Occupied Molecular Orbital
LUMO	-	Lowest Unoccupied Molecular Orbital
min	-	Minute
ml	-	Milliliter
MTCC	-	Microbial type culture collection
MTT	-	3-(5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide
PMR	-	Proton Magnetic Resonance
sec	-	Seconds
TLC	-	Thin Layer Chromatography
Δ E	-	Change in energy
µl	-	Micro liter
µm	-	Micro meter
^{1}H NMR	-	Proton Nuclear Magnetic Resonance spectroscopy

A	-	Absorbance
CFU	-	Colony Forming Unit
cm	-	Centimeter
div	-	Divison
DMSO	-	Dimethyl sulphoxide
DMSO-*d*6	-	Deuterated Dimethyl sulphoxide
lbs	-	Pounds
m	-	Meter
m/z	-	Mass / charge
M^+	-	Molecular Ion
MeOH	-	Methanol
mg	-	Milligram
mm	-	Millimeter
mol	-	Mole (s)
nm	-	Nanometer
ppm	-	Parts per million
UV	-	Ultra Violet
v	-	Volume
w	-	Weight
δ	-	Delta scale (chemical shift)
ε_{max}	-	Maximum absorbtivity
λ_{max}	-	Maximum wavelength

INTRODUCTION

1. INTRODUCTION

1.1. Pharmaceutical Chemistry vs Medicinal Chemistry:

Pharmaceutical chemistry is a science that makes use of the general laws of chemistry to study drugs, i.e., their proportion, chemical nature, composition, influence on an organism and studies of the physical and chemical properties of drugs, the method of quality control and the condition of their storage. The primary function of the pharmaceutical and medicinal chemist is still to design new drugs, with knowledge of principles of biochemical action of drug molecules.

Medicinal chemistry, according to burger, tries to be based on the ever increasing hope that biochemical rationales for drug discovery may be found a logical approach to study of drugs and their activities is the recognition of the basic principles behind the biochemical events leading to drug actions.

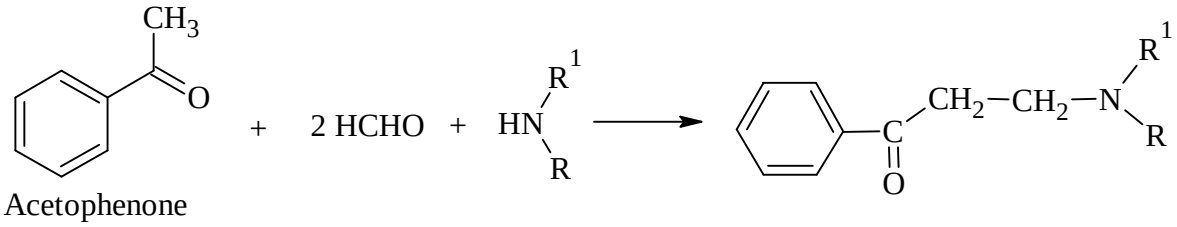
Chemical modifications of drug molecules to locate the number of series having optimal effects, and will probably continue to be a factor necessary to drug discovery. To establish the structure of the drug molecule the new inventions in physico-chemical properties such as X–rays analysis, UV, IR, NMR and Mass are immensely helpful for medicinal chemist. In the biochemical view knowledge of drug-receptor interaction, pharmacokinetics, advances in enzymology, have immensely helped medicinal chemist in hypothesizing the correct mechanism of action of drug molecules. The approach to practice the medicinal chemistry has developed from an empirical are involving organic synthesis of new compounds, based largely on modification of structures of known activity. (Donald J. Abraham., 2002)

1.2. Mannich Reaction and Mechanism:

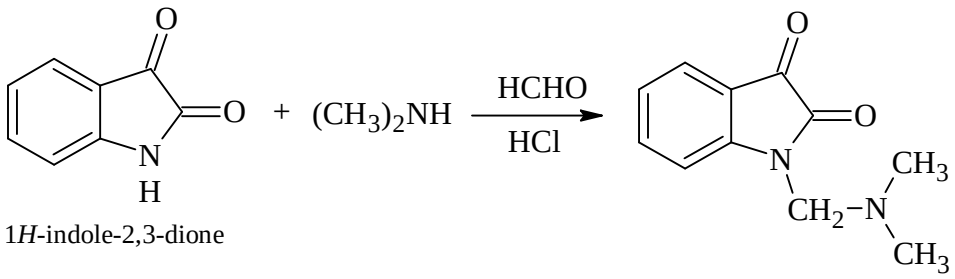
Mannich bases form a promising group of chemicals which may be a good source of potential candidates for future drugs. Our knowledge about their activities at the cellular and tissue level is, however, still rather limited. α, β – unsaturated ketones released by mannich bases exert their biological activities such as cytotoxicity and anti-fungal activity by reacting with the vital thiol groups in the living organisms.

Mannich reaction is one of the most important C-C bond forming reactions in organic synthesis for the preparation of secondary and tertiary amine derivatives. These amines are further used for the synthesis of many intermediates, biologically active and natural products such as alkaloids and polypeptides. The products of Mannich reaction are mainly amino carbonyl compounds and its derivatives that are used for the synthesis of amino alcohols, peptides and lactams and as precursors to optically active amino acids. (Suman Sahoo *et al.,* 2006)

The Mannich reaction has been reviewed by Blicke, Karbe, Nobles, Reichert, Thomson and others and had been mentioned in many books. There are two important types of mannich reactions, the C-type (Scheme 1) and the N-type (Scheme 2) reaction.



Scheme 1: C-type Mannich reaction

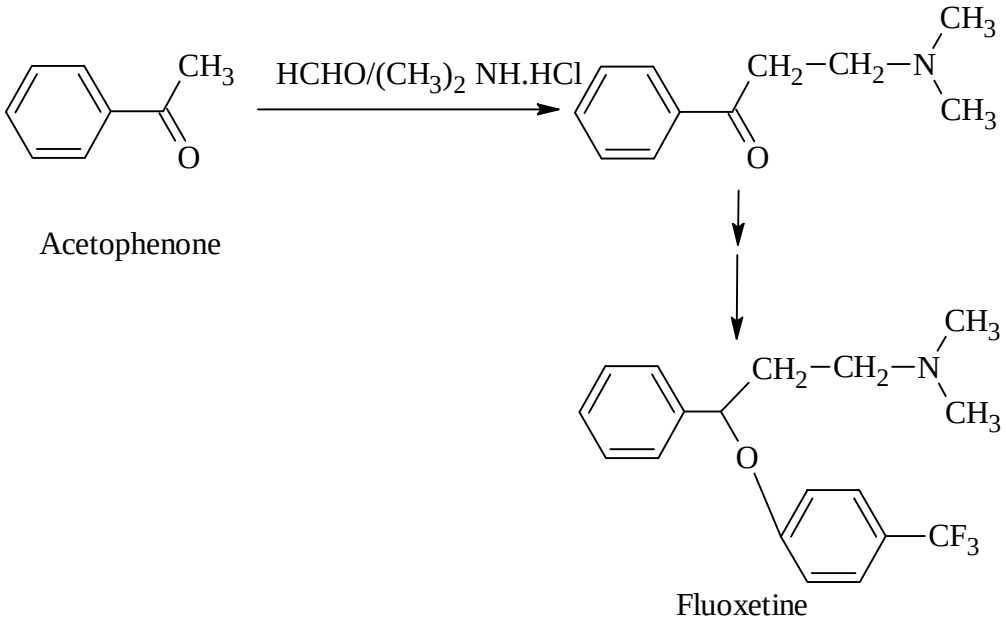


1*H*-indole-2,3-dione

Scheme 2: N-type Mannich reaction

1.3. Synthetic Utilities of Mannich bases:

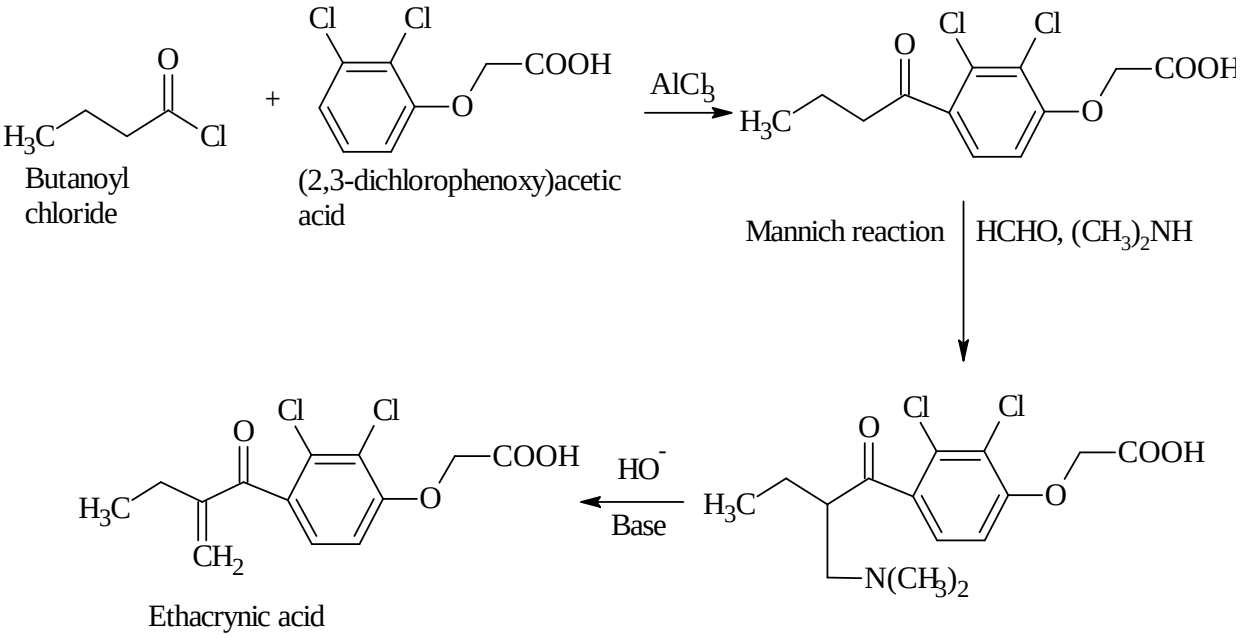
Mannich reactions have been used as a synthetic tool in the preparation of various therapeutic agents, fluoxetine, an antidepressant agent (Scheme 3), ethacrynic acid, a ceiling loop diuretics (Scheme 4), ranitidine, a H_2-receptor antagonist (Scheme 5), triprolidine, a H_1-receptor antagonist (Scheme 6) and trihexylphenidyl hydrochloride, an antispasmodic (Scheme 7).



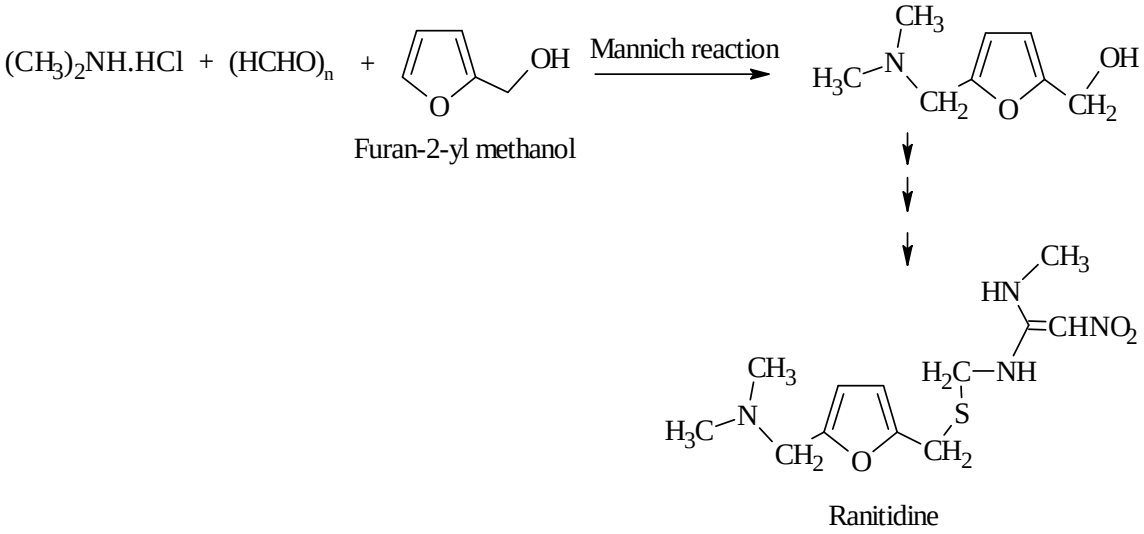
Acetophenone

Fluoxetine

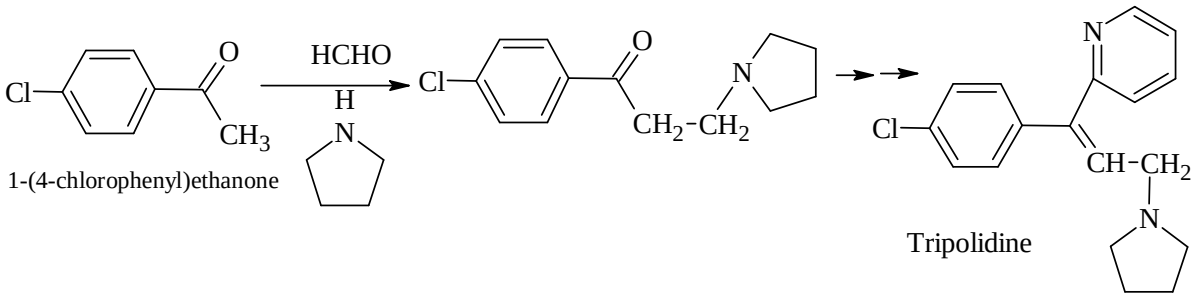
Scheme 3: Synthesis of Fluoxetine



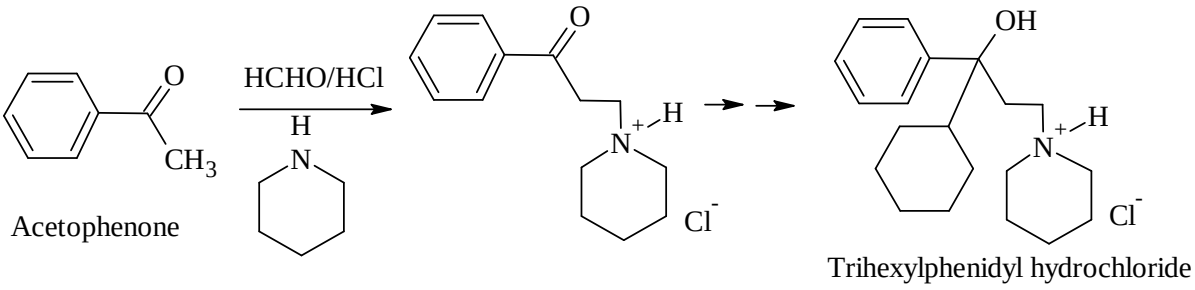
Scheme 4: Synthesis of Ethacrynic acid



Scheme 5: Synthesis of Ranitidine

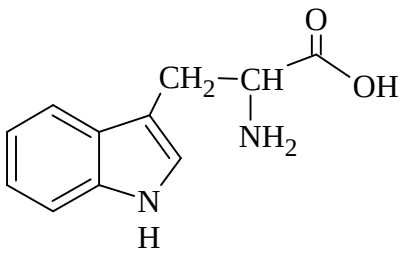


Scheme 6: Synthesis of Tripolidine

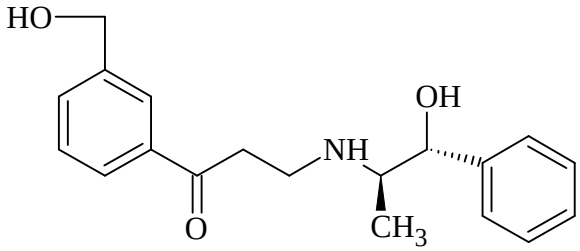


Scheme 7: Synthesis of Trihexylphenidyl hydrochloride

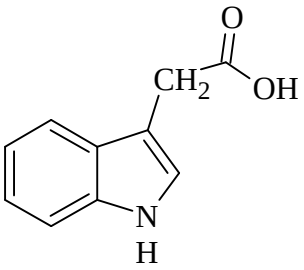
Compounds which are synthesized by Mannich reaction:



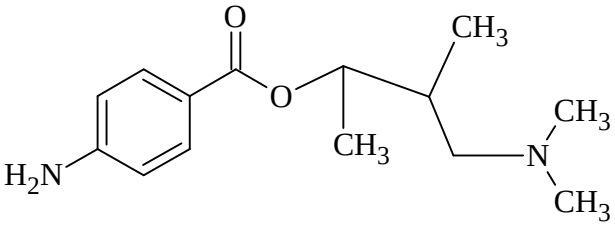
Heteroauxin (Growth promoting hormone)



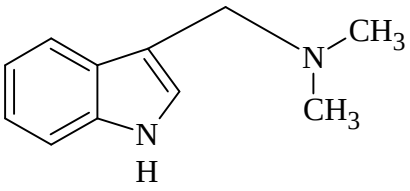
Oxyfedrine (Anti-anginal agent)



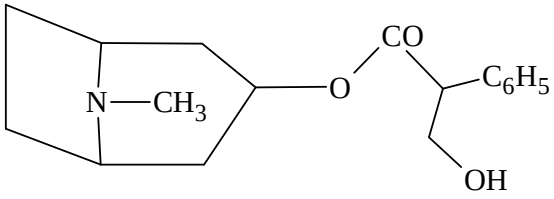
Tryptophan



Tutocaine (Local anesthetic)

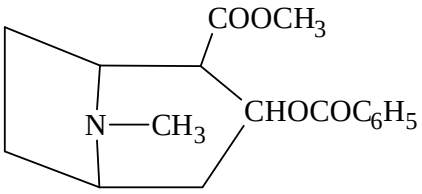


Gramine

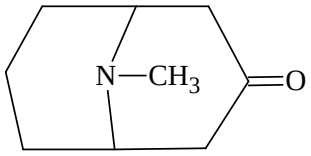


Atropine

(Present in plants and plays defensive role)



Cocaine



Pseudopelletierine

Fig 1: Compounds synthesized by Mannich reaction (Agarwal. O. P., 2008)

1.4. Mannich bases in Prodrug Design:

Mannich bases have been also explored, into the area of prodrugs. The hydrochloride salt of mannich base of carbamazepine (anticonvulsant) with dipropylamine is found to be more than 104 fold soluble in water than the parent drug. Following intramuscular administration in rats, higher and more rapidly appearing carbamazepine plasma levels were observed from aqueous solutions of the N-mannich base prodrug than from administering a suspension of the parent drug.

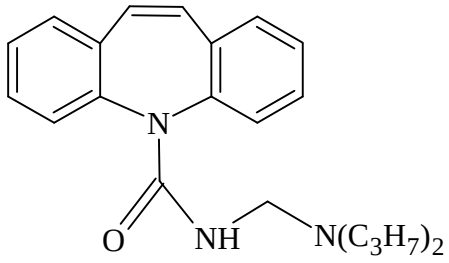


Fig 2: Carbamazepine mannich base

Hetacillin, formed by condensation of ampicillin with acetone, is regarded as cyclic N-mannich base. Sodium ampicillin in solution loses almost 10% of its activity in 1 h of room temperature at a concentration of 250 mg/ ml. Hetacillin, on the other hand, loses somewhat less than 10% activity in 6 h under identical conditions. The pharmacokinetics of oral and intravenous doses of ampicillin and hetacillin has been compared. Bioavailability studies showed 32% absorption from ampicillin capsules, while 42% absorption values from hetacillin capsules in non-fasting subjects.

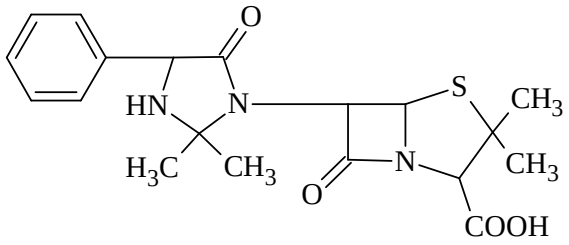


Fig 3: Hetacillin

Rolitetracyclin, a prodrug of tetracyclines prepared by reacting pyrrolidine and formaldehyde is very soluble in water whereas the parent drug tetracycline has low solubility in water.

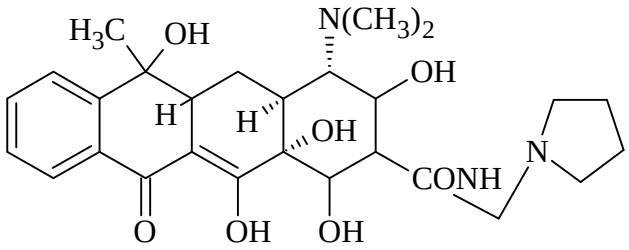


Fig 4: Rolitetracycline

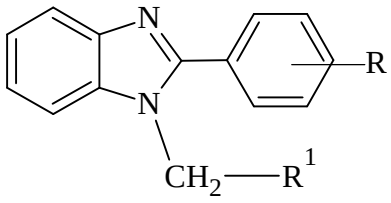
The concept of N-mannich base prodrug may be useful or improving the dissolution behavior of poorly soluble drugs in an effort of improves oral bioavailability of various NH – acidic compounds (chlozoxazone, phenytoin, sorbital, acetazolamide, chlorothiazide and allopurinol). In study, N-mannich bases with morpholine or piperidine were found to possess markedly greater (up to a factor of 2.000) intrinsic dissolution rates in comparison with the parent compounds. (Pandeya S.N. *et al.,* 2003)

LITERATURE

REVIEW

2. LITERATURE REVIEW

2.1. Rita Bamnela *et al.,* (2011) synthesized N-Mannich bases of 1-substituted methyl-2-(substituted phenyl) benzimidazole derivatives were characterized by elemental analysis and spectral data (IR and ^{1}H NMR). They have been evaluated for their antibacterial, anthelmintic and insecticidal activities against microbes, helminthes and insects. The below mentioned compounds (Fig–5) had exhibited significant anthelmintic activity while moderate to good antibacterial and insecticidal activities against helminthes, bacteria and insect selected as compared to standard drugs.

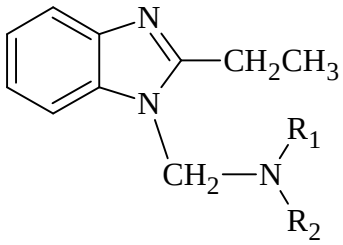


(Fig – 5)

Code. No	R	R_1
4a	4-NO_2	Piperazino
4b	4-NO_2	Diphenylamino
4c	4-NO_2	Morpholino
4d	4-NO_2	Diethanolamino
4e	4-NO_2	Imidazolo
4f	4-NO_2	Diethylamino
4g	4-OH	Piperazino
4h	4-OH	Diphenylamino
4i	4-OH	Morpholino
4j	2-OH	Piperazino
4k	2-OH	Diphenylamino
4l	2-Cl	Morpholino

2.2. Mariappan G. *et al.,* (2011) synthesized a novel series of 2-ethyl benzimidazole derivatives had been synthesized by the condensation of 2-ethyl benzimidazole with substituted primary and secondary amines. Their structures have been elucidated by UV-Vis, IR, ^{1}H NMR and Mass spectral data. Among the synthesized derivatives 1, 2, 8, 9 and 10 were found to have an effective anti-inflammatory response whereas compounds 2, 4, 6, 8 and 10 have potent analgesic response. There is no significant

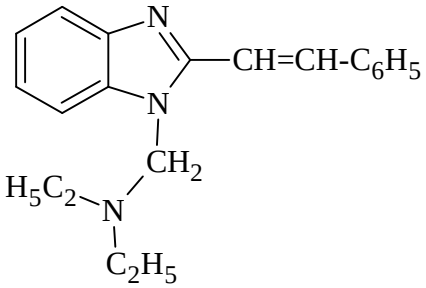
difference in bioactivity of benzimidazoles derived from secondary and primary amines.



(Fig – 6)

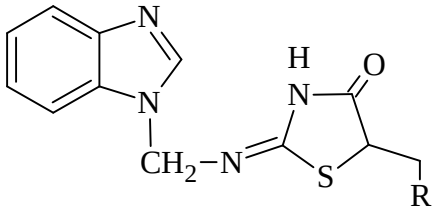
CODE	$-NR_1R_2$
1	Diethylamino
2	Piperidino
4	Diethanolamino
6	3-chloroanilino
8	3,4-dichloroanilino
9	4-fluroanilino
10	4-bromoanilino

2.3. Murugesan Sugumaran *et al.,* (2011) synthesized a series of mannich bases of 2-substituted benzimidazole were synthesized and characterized by their melting point and analytical thin layer chromatography. Structures of synthesized compounds were characterized by UV, IR, ^{1}H NMR and Mass spectral analysis. The synthesized compounds were screened for antibacterial and anthelminitic activity. The result showed one of the compounds (Mannich base of 2-styryl benzimidazole) had more potent activity in both antibacterial and anthelminitic activity.

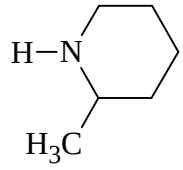
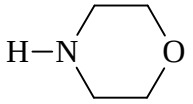


(Fig – 7)

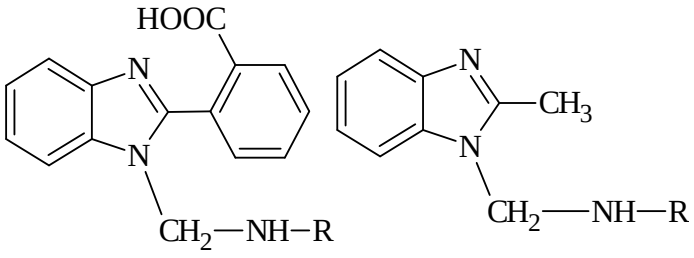
2.4. Pankaj Srivastava *et al.,* (2009) had synthesized mannich bases of 2-(benzimidazolyl aminomethyl) thiazolidin-4-one by amination at 5th position using formaldehyde and various secondary amines. The prepared compounds have been characterized by physico-chemical and spectral analysis and screened for their antibacterial activity. The below mentioned compounds (Fig – 8) were found to be remarkably effective compounds with respect to their inhibitory activity against gram positive bacteria *Bacillus subtilis* and gram negative bacteria, *Pseudomonas aerugenosa* and *E.coli*.



(Fig – 8)

Code. No	R
C_2	
C_4	

2.5. Kalirajan R. *et al.,* (2009) synthesized some new 2-substituted benzimidazoles like 2-(1H-benzo[d]imidazol-2-yl)benzoic acid (I) and 2-methyl benzimidazole (II) derivatives and they had characterized by TLC, elemental analysis, IR and ^{1}H NMR spectroscopy. The synthesized compounds were screened for their anti-inflammatory, antibacterial and antifungal activities and all the compounds have highly significant activity when compared with standard drug.

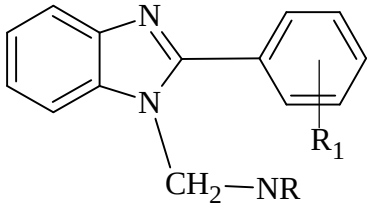


I II

(Fig – 9)

Code	NH – R
I a & II a	Morpholine
I b & II b	Piperidine
I c & II c	Diethyl amine
I d & II d	Diphenyl amine
I e & II e	Paracetamol
I f & II f	Acetanilide

2.6. Leonard J. T. *et al.*, (2007) synthesized a new series of substituted benzimidazoles as 1-(substituted methyl)-2-(substituted phenyl) benzimidazole and characterized by ^{1}H NMR, IR and elemental analyses. The compounds were evaluated for anti-inflammatory and antibacterial activity. The below mentioned compounds (Fig – 10) exhibited significant anti-inflammatory and antibacterial activities.

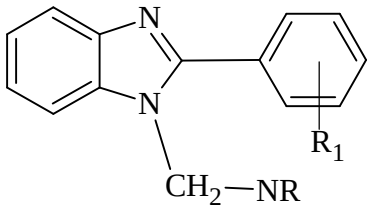


(Fig – 10)

Code. No	R_1	NR
1	3- NO_2	Morpholine
3	3- NO_2	Piperazine
4	3- NO_2	Imidazole
7	3- NO_2	4-Methyl piperazine
9	2- NH_2	Piperazine
10	2- NH_2	Piperidine
11	2- NH_2	Diethyl amine
12	2, 4-diCl	Piperazine

2.7. Leonard J. T. *et al.*, (2006) synthesized a new series of substituted benzimidazoles as 1-(substituted methyl)-2-(substituted phenyl) benzimidazoles and characterized by IR, ^{1}H NMR and elemental analysis. The compounds (Fig – 11) were evaluated for anti-inflammatory and antibacterial activity. In the anti-inflammatory study, the compounds (4, 5, 6 and 11) were produced good anti-inflammatory activity. In the

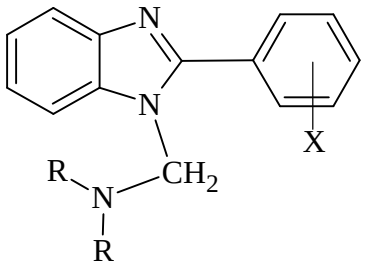
antibacterial evaluation, the compounds (3, 6, 8 and 12) were produced good antibacterial activity.



(Fig – 11)

Code. No	NR	R_1
3	4-Cl	Piperazine
4	4-Cl	Imidazole
5	4-Cl	Diphenylamine
6	4-Cl	Dimethylamine
8	4-Cl	Diethylamine
11	4-NH_2	Diphenylamine
12	4-NH_2	4-methyl piperazine

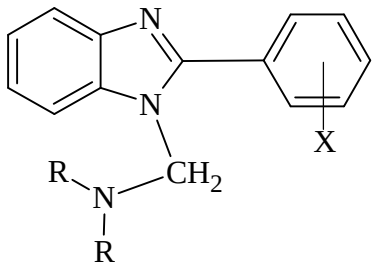
2.8. Mohamed Al Messmary *et al.,* (2010) synthesized a number of 2-substituted benzimidazoles were prepared by reaction of substituted benzoic acid with O-phenylenediamine, and then the products obtained were treated with secondary amines in the presence of formaldehyde in order to synthesized mannich bases. The synthesized products were characterized by physical and spectral analysis.



(Fig – 12)

X	Code	R
o-OH	II a	CH_3
p-OH	II b	CH_3
o-Cl	II c	CH_2CH_3
p-Cl	II d	CH_2CH_3
o-NO_2	II e	C_6H_5
p-NO_2	II f	C_6H_5

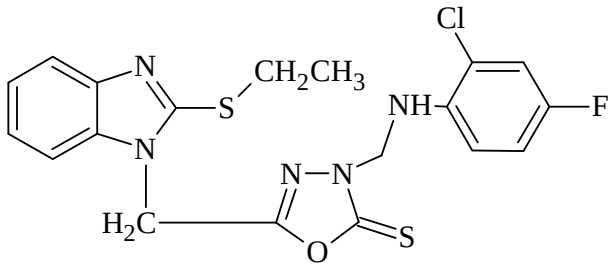
2.9. Mohamed G Elerafi *et al.,* (2010) synthesized a new series of substituted benzimidazoles of 1-(substituted methyl)-2-substituted phenyl benzimidazoles. The final products (Fig – 13) were characterized by IR, ^{1}H NMR spectra and elemental analysis.



(Fig – 13)

X	Code	R
H	II a	CH_3
p-CH_3	II b	CH_3
p-CH_3	II c	CH_2CH_3
o-OCH_3	II d	CH_2CH_3
o-OCH_3	II e	Morpholine
o-NH_2	II f	Piperidine

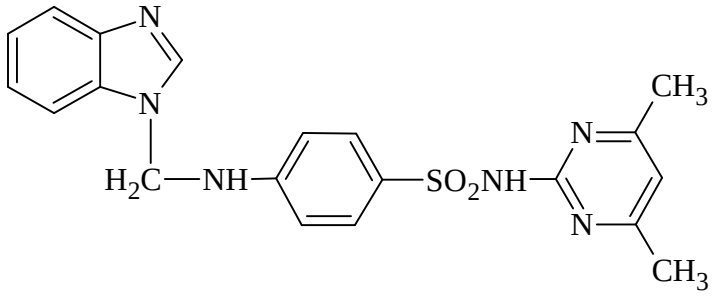
2.10. Vijayaraghavan S. *et al.,* (2009) synthesized a novel series of mannich bases by the reaction of 5-{2-(ethylthio)-1H–benzimidazole–1-yl}-methyl-1, 3, 4 – oxadiazole –2-thione with formaldehyde and appropriate amines. The final products were characterized by IR, ^{1}H NMR spectra and elemental analysis. The antibacterial screening against *S. aureus, E. coli* and *P. aeruginosa* at three different concentrations revealed that compound containing 2-chloro-4-flurophenyl substituted derivative (Fig – 14) showed significantly active.



(Fig – 14)

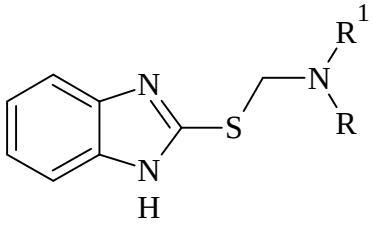
2.11. Periyasamy Selvam *et al.,* (2010) synthesized a series of novel N-substituted benzimidazole derivatives and screened anti-viral activity against a HIV-1 and HIV-2 in MT-4 cells and also cytotoxicity activity studied by MTT assay method. The novel compounds were synthesized through modifying the N-1 hydrogen of benzimidazole moiety with the substitution of sulphanilamide by mannich reaction. The final

synthesized products were characterized by means of IR and PMR spectra. Benzimidazole derivative (Sulphadimidine) had inhibited the replication of HIV-1 and 2 (EC_{50} = 35.40 µg/ ml and CC_{50}>125 µg/ ml) in MT-4 cells.

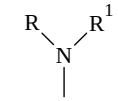
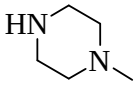
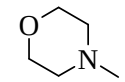
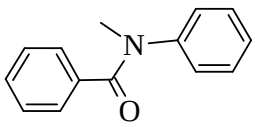


(Fig – 15)

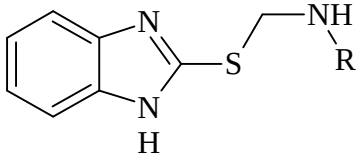
2.12. Anandarajagopal K. *et al.*, (2010) synthesized a novel series of 2-mercapto benzimidazole derivatives by mannich reaction from 2-mercapto benzimidazole by reaction with compounds having secondary amine and formaldehyde. The purity of the synthesized compounds was checked by melting point and TLC and their structure was established by various analytical techniques such as IR and ^{1}H NMR spectral studies. The final products were evaluated by anti-inflammatory, antimicrobial and anticonvulsant activities. The below mentioned compounds 1E, 1F and 1I were exhibited excellent anticonvulsant activity and also exhibited significant anti-inflammatory and antimicrobial activities.



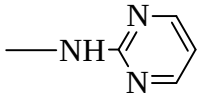
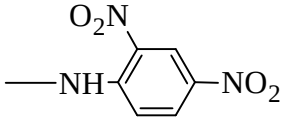
(Fig – 16)

Code	
1E	
1F	
1I	

2.13. Gigani Yaseen *et al.,* (2010) synthesized some 'best fit' 2MBI derivatives (Mannich bases) and evaluated by *in vitro* antimicrobial assay using paper disc method. Dihydrofolate reductase (DHFR) is the important target for antimicrobial drugs belonging to the class of anti-metabolites as the enzyme plays an important role in the de novo purine synthesis. Since 2-mercapto benzimidazole (2MBI) shares structural similarity with purine nucleotides. The structures were reported the *in silico* screening to obtain best fit molecules as DHFR inhibitors. The structures of these molecules (Fig – 17) were elucidated by Infrared and they were subjected for *in vitro* antimicrobial activity against gram positive and gram negative bacteria. The compounds containing AMP and DHH were most potent activity observed when compared with ciprofloxacin as standard at concentration 200 µg/ ml.

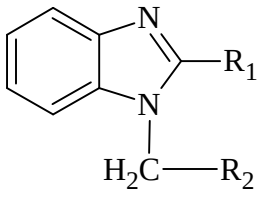


(Fig – 17)

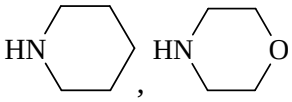
Code	R=
AMP	
DHH	

2.14. Philip Jesudason E. *et al.,* (2009) synthesized a novel series of N-mannich bases of benzimidazole derivatives and characterized by ^{1}H NMR, IR spectral studies and elemental analysis. The compounds were screened for analgesic and anti-inflammatory activity. Corneal permeability and quantum chemical calculations were performed to correlate the hydrogen bonding ability with permeability and activity. The energies of the highest occupied molecular orbital (HOMO) and the lowest

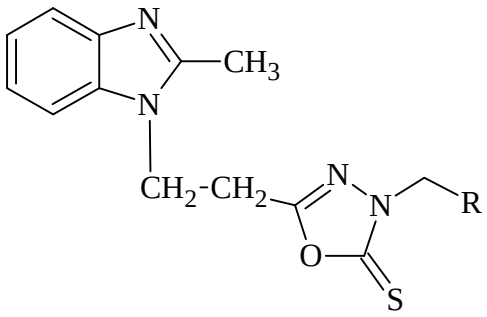
unoccupied molecular orbital (LUMO) were correlated with pharmacological activity. The semi-empirical PM3 calculations (quantum chemical calculations) revealed that E_{LUMO} and energy gap ΔE were capable of accounting for the high *in vitro* bovine corneal permeability and activity of the compounds.



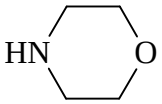
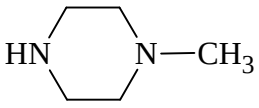
(Fig – 18)

R_1	**R_2**
H	$-N(CH_3)_2$, $-N(C_2H_5)_2$
CH_3	$-N(C_6H_5)_2$
$-CH=CH-C_6H_5$	$-N(C_2H_5)_2$, 

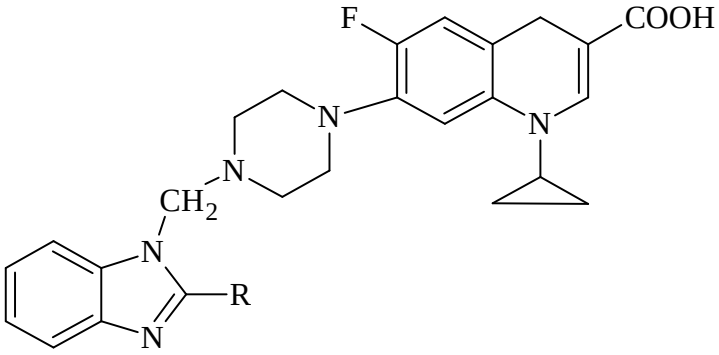
2.15. Afaf H. El-masry *et al.,* (2000) synthesized a new series of 3-(2-methyl benzimidazole-1-yl) propanoic acid hydrazide reacts with CS_2/KOH give oxadiazole derivative from this mannich base were synthesized. The synthesized compounds were characterized by IR, ^{1}H NMR and elemental analysis. The products (Fig – 19) were screened for antimicrobial activity.



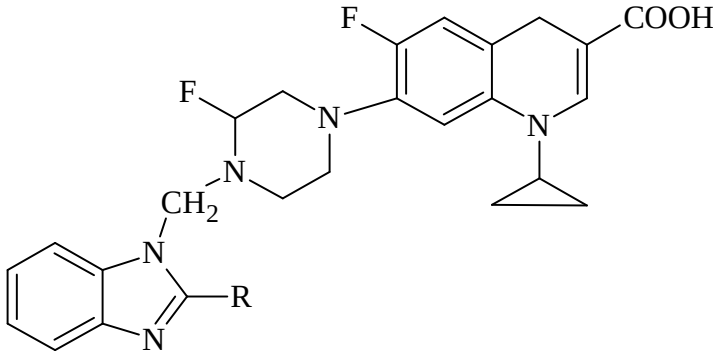
(Fig – 19)

R
$-N(C_2H_5)_2$



2.16. Jubie S. *et al.,* (2010) had synthesized a series of mannich bases of ciprofloxacin and norfloxacin with various benzimidazoles. The compounds were confirmed by physical parameters (solubility and melting point), TLC and at last spectroscopic methods (IR, NMR). The final compounds were screened for their antibacterial and antifungal activity by cup-plate method. All the benzimidazole substituted norfloxacin derivatives (NF_1, NF_2 and NF_3) and ciprofloxacin derivatives (CF_1, CF_2 and CF_3) were exhibit significant activity against *Candida albicans* at 50, 100 µg/ ml. The benzimidazoles showed mild antibacterial activites and significant antifungal activities.



Mannich base ($CF_1 – CF_3$)



Mannich base ($NF_1 – NF_3$)

R = H, CH_2CH_3, $CH_2CH_2CH_3$

(Fig – 20)

AIM AND PLAN OF WORK

3. AIM AND PLAN OF WORK

3.1. Aim:

In the present century due to the advancement and changes in the culture and life style, new diseases are being existed among the human population that the search for better dugs still necessary. Synthesis, new more than ever, is a vital and interesting part of organic chemistry. Organic synthesis has, rightly commanded a good deal of attention from researchers who have to choose from different arrays of synthetic methods. Discovery of new drugs that is therapeutically useful and goes into clinics is a life time dream for medicinal chemist. Carbocyclic (or) heterocyclic ring systems comprise the core of chemical structures of the vast majority of therapeutic agents. The exploitation of a simple molecule with different functionalities for the synthesis of heterocyclic compounds is a worthwhile contribution in the chemistry of heterocycles. Heterocyles dominate medicinal chemistry – the majorities of drugs are heterocyclic or have heterocyclic structural compounds. (Anandarajagopal K., *et al*., 2010 and Ganiyat K. Oloyede., *et al*., 2011)

Benzimidazoles are remarkably effective compounds both with respect to their degree of virus inhibitory activity and favorable selectivity ratio. Several benzimidazole derivatives with N-1 substitution showed anti-viral activity against human cytomegalo virus and herpes simplex virus type-1. 2-substituted benzimidazole derivatives, one of the most important derivatives of benzimidazole are also known possess varies biological activities. The biological activities of these compounds depend upon the substitution on the benzimidazole at the N-1 (or) C-2 position. Since benzimidazole heterocyclic ring

system mimics the purine bases like adenine and guanine of nucleic acids, the N-1 substituted benzimidazoles may be incorporated into the viral nucleic acids by enzymatic process and subsequently can alter the structure and function of nucleic acids resulting in the inhibition of viral growth (Periyasamy Selvam., *et al*., 2010).

Over the past few decades, Mannich bases of heterocyclic molecules have been grabbing the attention of the synthetic chemists for their wide gamut of biological activities ranging from antibacterial, anti cancer, anti parkinson to anti convulsant, anti inflammatory, analgesic and anti-HIV (Vijayaraghavan S., *et al*., 2009, Tewari R.K., *et al*., 1991, Dimmock J.R., *et al*., 1992 and Pandeya S.N., *et al*., 2003). The Mannich reaction is one of such. It involves the introduction of single carbon atom by the reaction of an active methylene compound with formaldehyde and a primary amine or secondary amine. Mannich reaction is a simple addition step without final elimination, this presumably reflects the fact that $-^{+}NH\text{-}R_2$ is a poorer leaving group than $-^{+}OH_2$. The products of many mannich reactions are referred to as Mannich bases and are themselves useful synthetic intermediates.

Multi-component reactions constitute a major part in the present day organic synthesis with advantages ranging from lower reaction times, reproducibility and improve biologically active compounds. It has been observed that the presence of two (or) more heterocyclic moieties fused or linked enhance the biological profile of drug molecules by many folds. Mannich reaction has been employed in the synthesis of many useful chemical substances of medicinal and industrial interest.

In the present research, we decided to synthesize 2-substituted benzimidazole derivatives by conventional method. Further the study will extended to introduce sulphanilamide and piperazine group substitution on N-1 position of 2-substituted benzimidazole by Mannich reaction and to screen the newly synthesized compounds for their antibacterial and antifungal activity.

3.2. Plan of the work:

- To synthesize 2-substituted benzimidazole derivatives and their corresponding N- mannich bases.
- To determine the physical properties such as percentage yield, melting point, R_f value, colour, solubility and nature of all the synthesized compounds ($SR_1 – SR_9$).
- To determine and characterize the spectral data such as UV spectroscopy, FT-IR, ^1H NMR and Mass spectra of all the synthesized compounds ($SR_1 – SR_9$).
- To evaluate the biological activity such as antibacterial and antifungal activity of synthesized compounds ($SR_4 – SR_9$).

EXPERIMENTAL

4. EXPERIMENTAL

4.1. Materials and Instruments:

The melting points were recorded in open capillaries on Elico melting point apparatus and were uncorrected. Spectral analysis were performed in the Sophisticated Analytical Instrumentation Facility, Indian Institute of Technology, Chennai, using ^{1}H NMR (Bruker – NMR 500MHz) Spectrometer and Mass (JEOL GC mate) spectrometer, in Ideal Analytical and Research Institution, Puducherry, using FT-IR (Thermo Fischer – Nicolet iS5) and Adhiparasakthi College of Pharmacy for UV-Visible Spectrometer (Double beam UV Spectrometer – SHIMADZU -1700).

In ^{1}H NMR, chemical shifts were reported in δ values using MeOD and DMSO-*d*6 are as a solvents and tetramethylsilane (TMS) as an internal standard with number of protons, multiplicities (s-singlet, d-doublet, t-triplet, q-quartet and m-multiplet) in the solvent indicated. IR spectra were recorded as KBr pellets and intensities mentioned as s-strong, m-medium and w-weak.

The reagents and solvents were commercially available (Rankem, SD fine, Loba and Fluka) and of synthetic grade. Glassware was oven or flame - dried for moisture sensitive reactions. When necessary, solvents and reagents were dried in prior to use. Solution or extracts in organic solvents were dried over anhydrous sodium sulphate or fused calcium chloride before evaporation to under vacuum using rotary evaporator. Analytical samples were dried in vacuum and they were free of significant impurities on TLC. For antimicrobial activity by using two gram positive bacteria (*Staphylococcus aureus* MTCC 740 and *Bacillus subtilis* MTCC 121) and two gram negative bacteria

(Escherichia coli MTCC 1302 and *Pseudomonas aeruginosa* MTCC 741) and two fungal organisms (*Candida albicans* ATCC 24433 and *Trichophyton rubrum* ATCC 2327) were collected from Microbial Resources Division, Kings Institute's of Preventive Medicine, Guindy, Chennai. The nutrient agar medium and sabouraud's dextrose agar medium were purchased from HI Laboratories Ltd., Mumbai, India.

4.2. Methodology:

Monitoring of Synthetic Reaction Procedures:

Established synthetic procedures were employed for synthesis of compounds SR_1 to SR_9 and the reactions were monitored by Thin Layer Chromatography (TLC) employing 6'' X 2'' plates coated with 0.25 mm thick layer of silica gel (pre-activation by heating at 110° C for one h). Solvent systems of varying polarity ranging from methanol to water mixtures (9.5:0.5, 9:1, 8.5:1.5, 8:2 and 7:3) were used to monitor the reactions. The plates were visualized in an iodine chamber.

Purification Techniques:

Recrystallization: The crude products were recrystallized by charcoal treatment in appropriate solvent. Single solvent was used wherever possible and solvent mixtures were used if necessary.

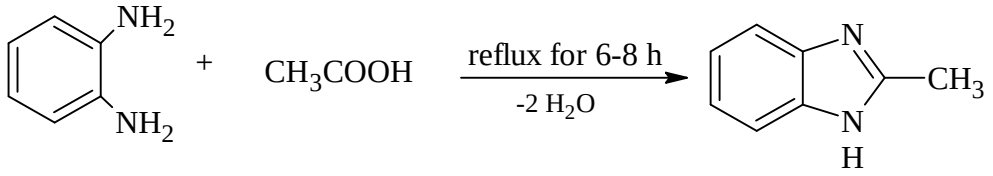
Authentication of Chemical Structures and Purity of Compounds:

Chemical structure of products and their purity were ascertained by thin layer chromatography, UV-Visible spectrometer, melting point and various spectral techniques including Fourier Transform Infra Red Spectroscopy, Nuclear Magnetic Resonance Spectroscopy and Mass Spectroscopy.

4.3. Synthesis of compounds:

4.3.1. Synthesis of 2–methyl-benzimidazole: (SR_1)

13.5 g (0.125 mol) of O-phenylene diamine was placed in a 250 ml of round bottom flask and added 10.2 g (0.17 mol) of acetic acid. The mixture was heated on a water bath at 100° C for 6-8 h, cooled and added 10% sodium hydroxide solution slowly with constant rotation of the flask, until the mixture was just alkaline to litmus. The crude benzimidazole derivative was filtered at the pump, and then washed with ice cold water, drained well and washed again with 25 ml of cold water. The crude product was dissolved in 200 ml of boiling water; 2 g of decolorizing carbon was added and digested for 15 min. The product was filtered rapidly at the pump through preheated buchner funnel and flask. The filtrate was cooled to about 10° C and the filtered product of 2-methyl-benzimidazole was again washed with 25 ml of cold water, dried at 100° C and weighed. (Vogel's 2006, Ahuluwalia V.K. *et al*., 2000, Ansari K.F. *et al*., 2009)

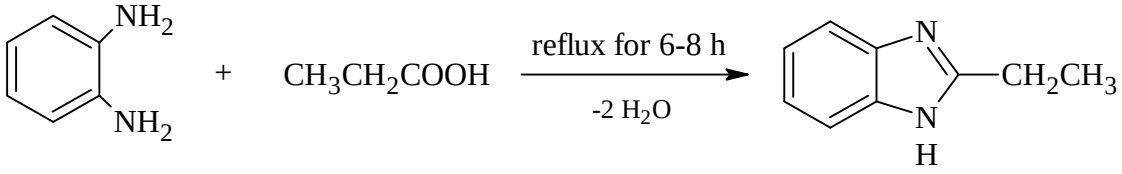


O-phenylene diamine acetic acid 2-methyl- benzimidazole

Scheme 8: Synthesis of 2-methyl-benzimidazole

4.3.2. Synthesis of 2-ethyl-benzimidazole: (SR_2)

13.5 g (0.125 mol) of O-phenylene diamine was placed in a 250 ml of round bottom flask and added 14.96 g (0.17 mol) of propionic acid. The mixture was heated on a water bath at 100° C for 6-8 h, cooled and added 10% sodium hydroxide solution slowly with constant rotation of the flask, until the mixture was just alkaline to litmus. The crude benzimidazole derivative was filtered at the pump, and then washed with ice cold water, drained well and washed again with 25 ml of cold water. The crude product was dissolved in 200 ml of boiling water; 2 g of decolorizing carbon was added and digested for 15 min. The product was filtered rapidly at the pump through preheated buchner funnel and flask. The filtrate was cooled to about 10° C and the filtered product of 2-ethyl-benzimidazole was again washed with 25 ml of cold water, dried at 100° C and weighed.

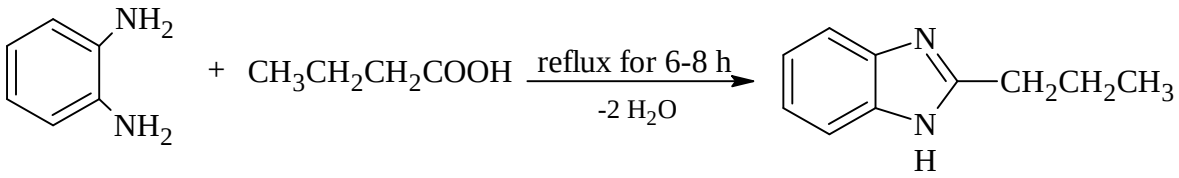


O-phenylene diamine propionic acid 2-ethyl- benzimidazole

Scheme 9: Synthesis of 2-ethyl-benzimidazole

4.3.3. Synthesis of 2-propyl-benzimidazole: (SR_3)

13.5 g (0.125 mol) of O-phenylene diamine was placed in a 250 ml of round bottom flask and added 17.34 g (0.17 mol) of butyric acid. The mixture was heated on a water bath at 100° C for 6-8 h, cooled and added 10% sodium hydroxide solution slowly with constant rotation of the flask, until the mixture was just alkaline to litmus. The crude benzimidazole derivative was filtered at the pump, and then washed with ice cold water, drained well and washed again with 25 ml of cold water. The crude product was dissolved in 200 ml of boiling water; 2 g of decolorizing carbon was added and digested for 15 min. The product was filtered rapidly at the pump through preheated buchner funnel and flask. The filtrate was cooled to about 10° C and the filtered product of 2-propyl-benzimidazole was again washed with 25 ml of cold water, dried at 100° C and weighed.

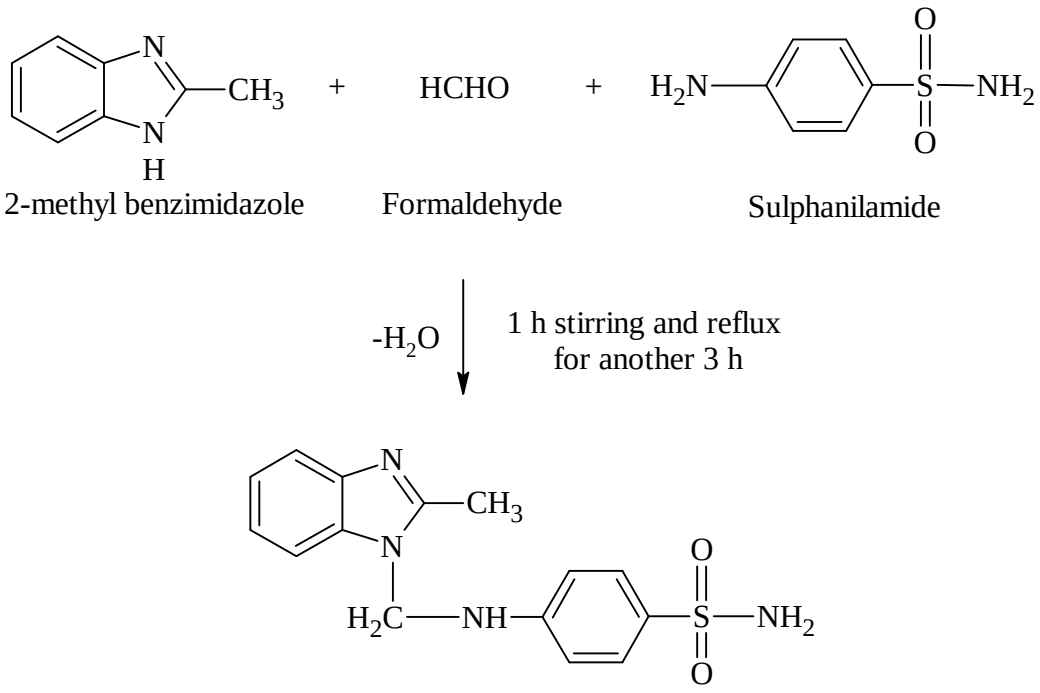


O-phenylene diamine butyric acid 2-propyl- benzimidazole

Scheme 10: Synthesis of 2-propyl-benzimidazole

4.3.4. Synthesis of 1-((sulphanilamido) methyl)-2-methyl-benzimidazole (SR_4):

To the 15 ml of methanolic solution, 0.66 g (0.005 mol) of 2-methyl benzimidazole was added to 0.86 g (0.005 mol) of sulphanilamide slowly with constant stirring under rigorous ice cooling. The reaction mixture was cooled well and 0.138 ml (0.005 mol) of formaldehyde solution (37% v/v) was added slowly with constant stirring. The reaction mixture was then adjusted to the pH of 3.5 with hydrochloric acid. The reaction mixture was kept in efficient ice cooling for half an hour to avoid losses of formaldehyde and then refluxed on water bath up to 3 h. The reflux time was dependent upon the sulphonamide chosen. After refluxing, the refluxed mixture was cooled at 0° C for 4 days, when crystallized product was obtained, which was recrystallized with dry distilled ethanol and DMF. (Sheela Joshi *et al.*, 2005)

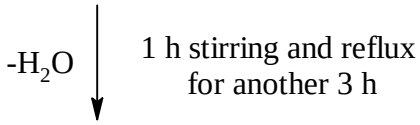


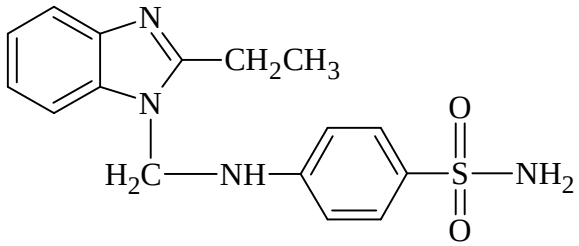
Scheme 11: Synthesis of 1-((sulphanilamido) methyl)-2-methyl-benzimidazole

4.3.5. Synthesis of 1-((sulphanilamido) methyl)-2-ethyl-benzimidazole (SR_5):

To the 15 ml of ethanolic solution, 0.73 g (0.005 mol) of 2-ethyl benzimidazole was added to 0.86 g (0.005 mol) of sulphanilamide slowly with constant stirring under rigorous ice cooling. The reaction mixture was cooled well and 0.138 ml (0.005 mol) of formaldehyde solution (37% v/v) was added slowly with constant stirring. The reaction mixture was then adjusted to the pH of 3.5 with hydrochloric acid. The reaction mixture was kept in efficient ice cooling for half an hour to avoid losses of formaldehyde and then refluxed on water bath up to 3 h. The reflux time was dependent upon the sulphonamide chosen. After refluxing, the refluxed mixture was cooled at 0° C for 4 days, when crystallized product was obtained, which was recrystallized with dry distilled ethanol and DMF.

2-ethyl benzimidazole Formaldehyde Sulphanilamide





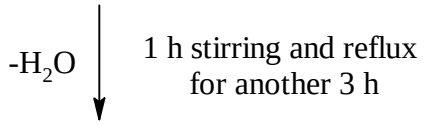
N-Mannich base of 2-ethyl benzimidazole

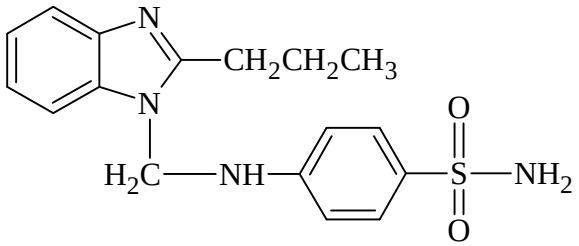
Scheme 12: Synthesis of 1-((sulphanilamido) methyl)-2-ethyl-benzimidazole

4.3.6. Synthesis of 1-((sulphanilamido) methyl)-2-propyl-benzimidazole (SR_6):

To the 15 ml of ethanolic solution, 0.80 g (0.005 mol) of 2-propyl benzimidazole was added to 0.86 g (0.005 mol) of sulphanilamide slowly with constant stirring under rigorous ice cooling. The reaction mixture was cooled well and 0.138 ml (0.005 mol) of formaldehyde solution (37% v/v) was added slowly with constant stirring. The reaction mixture was then adjusted to the pH of 3.5 with hydrochloric acid. The reaction mixture was kept in efficient ice cooling for half an hour to avoid losses of formaldehyde and then refluxed on water bath up to 3 h. The reflux time was dependent upon the sulphonamide chosen. After refluxing, the refluxed mixture was cooled at 0° C for 4 days, when crystallized product was obtained, which was recrystallized with dry distilled ethanol and DMF.

2-propyl benzimidazole Formaldehyde Sulphanilamide



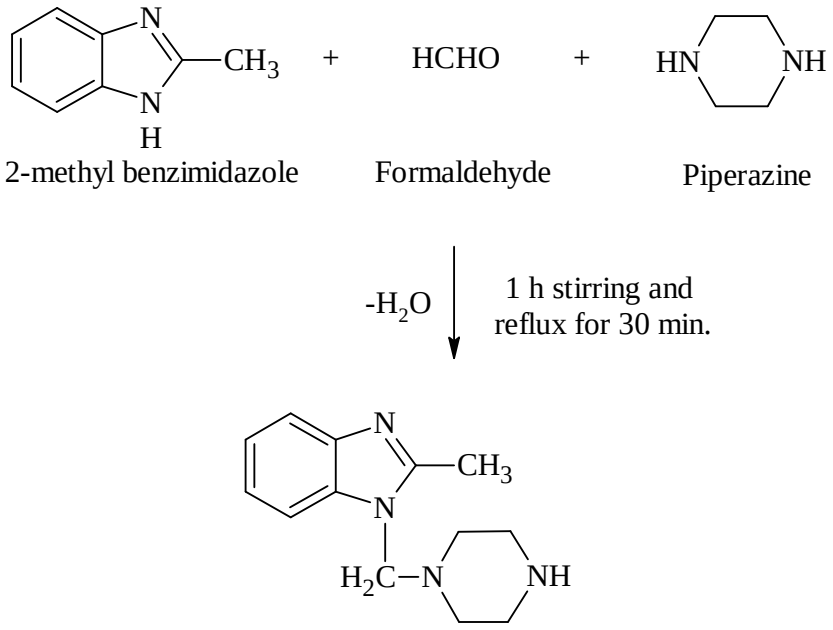


N-Mannich base of 2-propyl benzimidazole

Scheme 13: Synthesis of 1-((sulphanilamido) methyl)-2-propyl-benzimidazole

4.3.7. Synthesis of 1-((pipearzino) methyl)-2-methyl-benzimidazole (SR_7):

To a solution 1.32 g (0.01 mol) of 2-methyl benzimidazole in 15 ml of ethanol, 0.86 g (0.01 mol) of piperazine and 0.30 ml (0.01 mol) of formaldehyde solution (37% v/v) were added slowly with constant stirring under rigorous ice cold condition. The reaction mixture was kept in efficient ice cooling for half an hour to avoid losses of formaldehyde and then the reaction mixture was refluxed on water bath up to 30 min. The refluxed mixture was cooled at 0° C for 2-3 days in deep freeze. When crystallized product was obtained, filtered and dried. The product obtained was purified by recrystallized with dry distilled ethanol. (Rita Bamnela *et al.*, 2011)



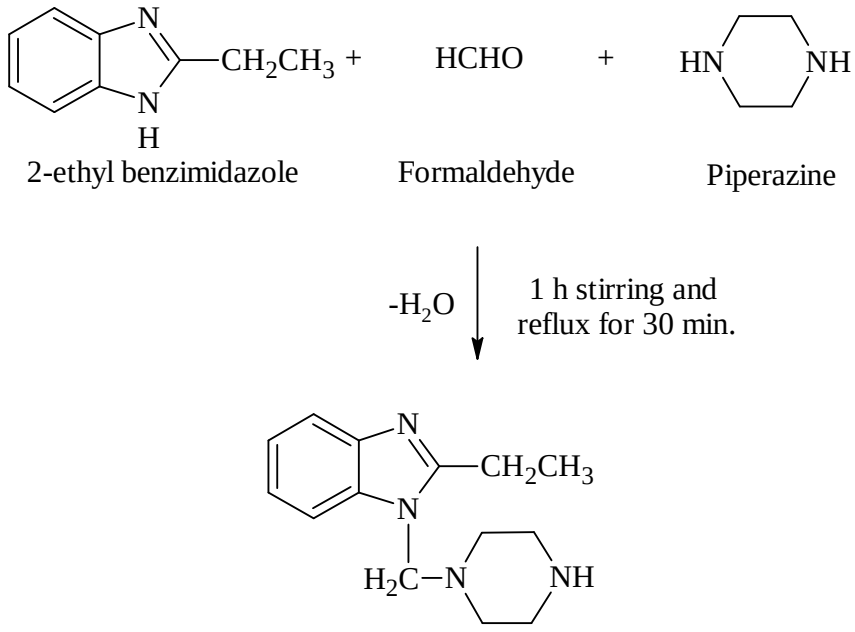
2-methyl benzimidazole Formaldehyde Piperazine

N-Mannich base of 2-methyl benzimidazole

Scheme 14: Synthesis of 1-((piperazino) methyl)-2-methyl-benzimidazole

4.3.8. Synthesis of 1-((pipearzino) methyl)-2-ethyl-benzimidazole (SR_8):

To a solution 1.46 g (0.01 mol) of 2-ethyl benzimidazole in 15 ml of ethanol, 0.86 g (0.01 mol) of piperazine and 0.30 ml (0.01 mol) of formaldehyde solution (37% v/v) were added slowly with constant stirring under rigorous ice cold condition. The reaction mixture was kept in efficient ice cooling for half an hour to avoid losses of formaldehyde and then the reaction mixture was refluxed on water bath up to 30 min. The refluxed mixture was cooled at 0° C for 2-3 days in deep freeze. When crystallized product was obtained, filtered and dried. The product obtained was purified by recrystallized with dry distilled ethanol.



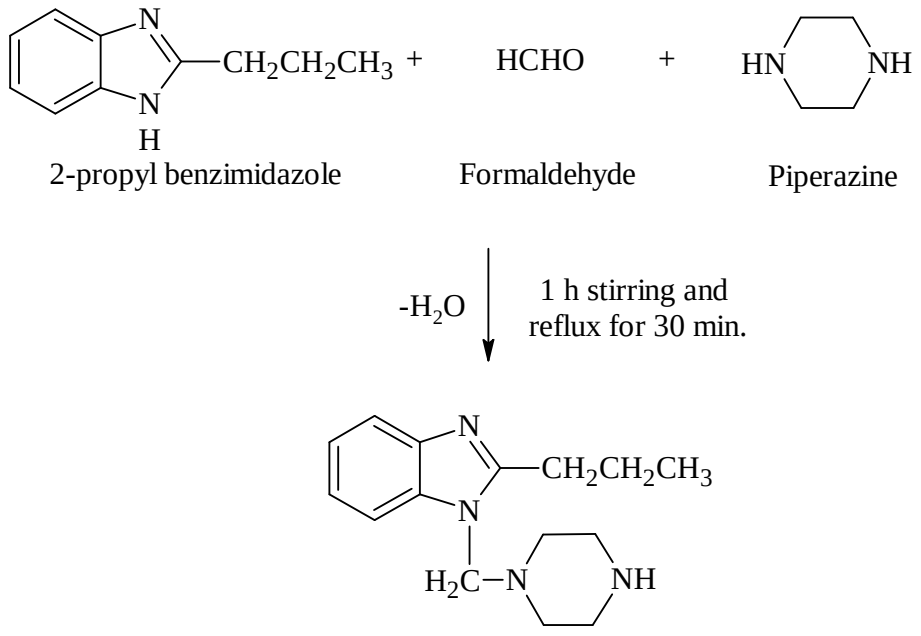
2-ethyl benzimidazole Formaldehyde Piperazine

N-Mannich base of 2-ethyl benzimidazole

Scheme 15: Synthesis of 1-((piperazino) methyl)-2-ethyl-benzimidazole

4.3.9. Synthesis of 1-((pipearzino) methyl)-2-propyl-benzimidazole (SR_9):

To a solution 1.60 g (0.01 mol) of 2-propyl benzimidazole in 15 ml of ethanol, 0.86 g (0.01 mol) of piperazine and 0.30 ml (0.01 mol) of formaldehyde solution (37% v/v) were added slowly with constant stirring under rigorous ice cold condition. The reaction mixture was kept in efficient ice cooling for half an hour to avoid losses of formaldehyde and then the reaction mixture was refluxed on water bath up to 30 min. The refluxed mixture was cooled at 0° C for 2-3 days in deep freeze. When crystallized product was obtained, filtered and dried. The product obtained was purified by recrystallized with dry distilled ethanol.



2-propyl benzimidazole Formaldehyde Piperazine

N-Mannich base of 2-propyl benzimidazole

Scheme 16: Synthesis of 1-((piperazino) methyl)-2-propyl-benzimidazole

BIOLOGICAL

SCREENING

5. BIOLOGICAL SCREENING

5.1. Evaluation of *in vitro* antibacterial activity:

The *in vitro* anti bacterial study was carried out by using selected gastrointestinal tract infection (GIT) causing pathogens which includes two gram positive bacteria (*Staphylococcus aureus* MTCC 740 and *Bacillus subtilis* MTCC 121) and two gram negative bacteria (*Escherichia coli* MTCC 1302 and *Pseudomonas aeruginosa* MTCC 741). The synthesized compounds ($SR_4 - SR_9$) were tested for anti bacterial activity by disc diffusion method (Collin C.H., *et al.*, 1995). They were dissolved in DMSO and sterilized by filtering through 0.45 µm millipore filter. Final inoculum of 100 µl suspension containing 10^8 CFU/ ml of each bacterium was used. Nutrient agar medium was prepared and sterilized by an autoclave (121° C and 15 lbs for 20 min) and it transferred to previously sterilized petridishes (9 cm in diameter). After solidification, petriplates was inoculated with gram negative bacterial organisms *Escherichia coli* MTCC 1302, *Pseudomonas aeruginosa* MTCC 741 and gram positive bacterial organisms *Staphylococcus aureus* MTCC 740 and *Bacillus subtilis* MTCC 121 in sterile nutrient agar medium at 45° C under aseptic condition. Sterile Whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized compounds at concentrations of 25, 100 µg/ disc were placed in the organism-impregnated petri plates under sterile condition. The plates were left for 30 min to allow the diffusion of compounds at room temperature. Antibiotic disc of ciprofloxacin (100 µg/ disc) was used as positive control, while DMSO used as negative control. Then the plates were incubated for 24 h at 37 ± 1° C. The zone of inhibition (Table-2) was calculated by

measuring the minimum dimension of the zone of no microbial growth around the each disc.

5.2. Evaluation of *in vitro* anti fungal activity:

The synthesized compounds (SR_4 - SR_9) were tested for anti-fungal activity by disc diffusion method. They were dissolved in DMSO and sterilized by filtering through 0.45 µm millipore filter. Final inoculum of 100 µl suspension containing 10^8 CFU / ml of each fungus was used. Sabouraud's dextrose agar medium was prepared and sterilized by an autoclave (121° C and 15 lbs for 20 min) and transferred to previously sterilized petridishes (9 cm in diameter). After solidification, petriplates were inoculated with fungal organisms *Candida albicans* ATCC 24433 and *Trichophyton rubrum* ATCC 2327 in sterile sabouraud's dextrose agar medium at 45° C in aseptic condition. Sterile Whatmann filter paper discs (previously sterilized in U.V. lamp) were impregnated with synthesized compounds at a concentration of 25, 100 µg/ disc were placed in the organism-impregnated petri plates under sterile condition. The plates were left for 30 min to allow the diffusion of compounds at room temperature. Antibiotic disc of ketaconazole (100 µg/ disc) were used as positive control, while DMSO used as negative control. Then the plates were incubated for 48 h at 37±1° C for antifungal activity. The zone of inhibition (Table-3) was calculated by measuring the minimum dimension of the zone of no microbial growth around the each disc.

RESULTS

AND

DISCUSSION

6. RESULTS AND DISCUSSION

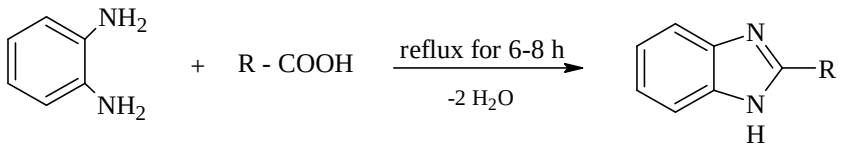
6.1. Synthetic Scheme:

The various strategies are available for the synthesis of benzimidazole and using a variety of starting material. In literature review, there are two general methods for the synthesis of 2-substituted benzimidazoles revealed that the condensation of O-phenylenediamine with carboxylic acid derivatives like anhydrides, ester, amides and acid chlorides to yield the corresponding the titled benzimidazole compound which often requires strong acidic conditions, and sometimes combines with very high temperatures or microwave irradiation. The other way involves a two-step procedure that includes the oxidative cyclo-dehydrogenation of schiff bases, which are often generated from the condensation of phenylenediamines and aldehydes. (Mazaahir Kidwai., *et al*., 2010; Sharma S., *et al*., 2009; Tanious F.A., *et al*., 2004; Fairley T.A., *et al*., 1993; Czarny A., *et al*., 1996; Raut C.N., *et al*., 2009; Nagata K., *et al*., 2003; Chakrabarty M., *et al*., 2006; Bahrami K., *et al*., 2007; Aliyan H., *et al*., 2009). A microwave-assisted method for the synthesis of 2- substituted benzimidazoles in the presence of alumina-methanesulfonic acid (AMA) was also reported. In recent years, solvent – free synthesis of benzimidazoles under microwave irradiation using $Yb(OTf)_3$, KSF clay, PPA, metal halide supported alumina and solid support have been reported.

In step I, compounds in which the aliphatic ring was directly attached to the second position of the benzimidazole ring were substituted by our scheme. In this method 2-substituted benzimidazoles were synthesized from O-phenylene diamine by reaction with mono aliphatic carboxylic acids. The reaction was neutralized with sodium

hydroxide solution and the crude benzimidazole derivatives (SR_1-SR_3) were isolated by filtration. (Goel. P. K., *et al.,* 2007*)*.

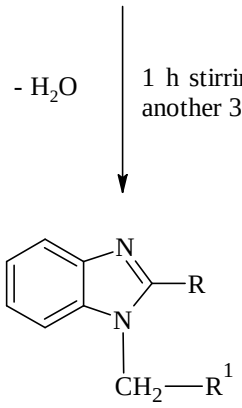
Step I:



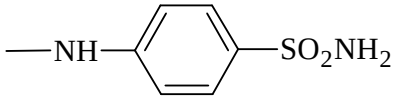
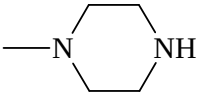
O-phenylene diamine carboxylic acid 2-substituted benzimidazole

Step II:

2-substituted benzimidazole formaldehyde primary (or) secondary amine



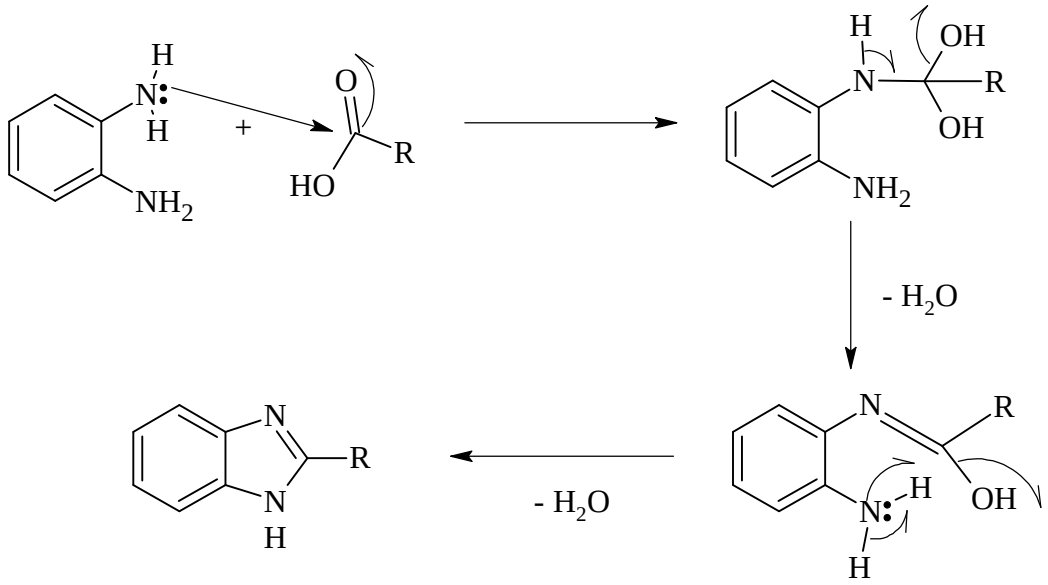
N-mannich base of 2-substituted benzimidazole

Code. No	R	R^1
SR_1	$-CH_3$	-
SR_2	$-CH_2CH_3$	-
SR_3	$-CH_2CH_2CH_3$	-
SR_4	$-CH_3$	
SR_5	$-CH_2CH_3$	
SR_6	$-CH_2CH_2CH_3$	
SR_7	$-CH_3$	
SR_8	$-CH_2CH_3$	
SR_9	$-CH_2CH_2CH_3$	

Scheme 17: Synthetic route of title compounds

The reaction is carried out according to the reaction, the first step involve the synthesis of benzimidazole derivatives, in step II, these compounds were treated with the primary or secondary amines in the presence of formaldehyde to obtain the corresponding N-mannich bases.

Mechanism of step I:



Scheme 18: Reaction mechanism for synthesis of 2- substituted benzimidazole

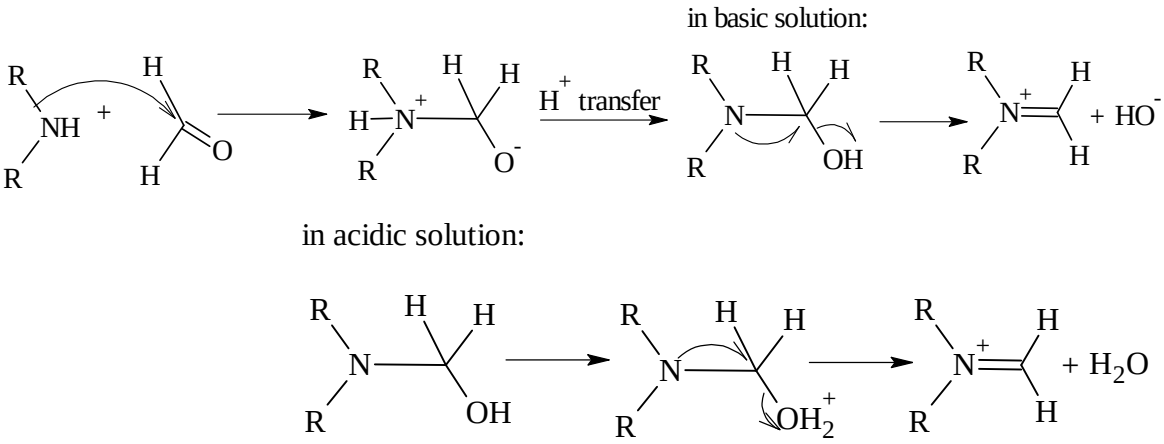
The Mannich reaction plays an essential role in the biosynthesis of important nitrogenous substances. In spite of the importance of the reaction, a satisfactory mechanism has not been elucidated. Several different mechanisms probably operate, depending upon pH and the nature of the active hydrogen of reactant (Eurckhalter J. H., *et al*., 1964).

This is the condensation between a compound containing at least one active hydrogen atom, formaldehyde and ammonia, a primary or a secondary amine (preferably as the hydrochloride). Compounds containing reactive hydrogen atom like ketones, acids

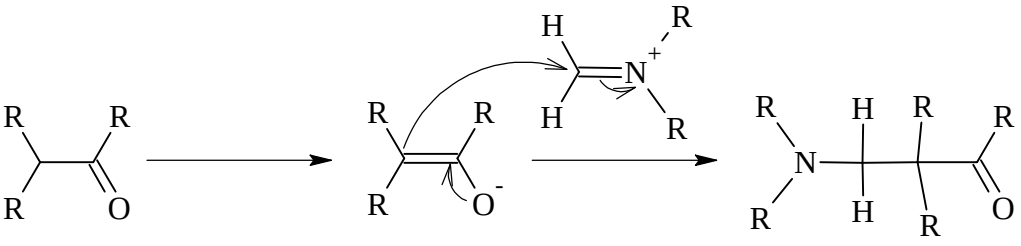
and their esters, phenols, furan, pyrrole, indole and their derivatives undergo this reaction very easily. The amine may be primary or secondary but the most convenient are secondary amines since they possess only one replaceable hydrogen atom (R_2NH). The product with primary amine is secondary which further reacts to give tertiary amine and formaldehyde is the most common component in the mannich reaction (Agarwal O.P., *et al*., 2008).

Mechanism of step II:

The first step in the mechanism is formation of an iminium ion from the amine (primary or secondary) and the formaldehyde:



In the next step, the carbonyl group forms an enolate (nucleophile) that attacks the iminium (electrophile):



Scheme 19: Mechanism of mannich reaction

The net result is that you have added an amino group in the beta position of a carbonyl compound. The reaction takes place usually under acidic conditions, which helps to generate the iminium intermediate (Abd El-Wareth A.O., *et al.*, 2006).

6.2. Interpretation of spectral data of synthesized compounds (SR_1-SR_9)

6.2.1 Spectral analysis of 2-methyl benzimidazole (SR_1):

Spectroscopy Data:

UV (MeOH): (Fig 22)

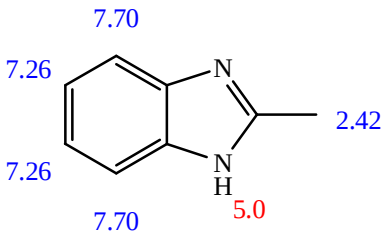
λ_{max} - 280.5 **(**ε_{max} 0.2668)

λ_{max} - 243.0 **(**ε_{max} 1.0140)

IR (KBr): (Fig 23)

Wave number (cm^{-1})	**Assignment**
3385 (s)	N-H stretching
3057 (w)	Aromatic (=C-H) stretching
2916 (m)	Aliphatic C-H stretching in CH_2
1624 (s)	C=C stretching
1591 (s)	C=N stretching
736 (s)	Aromatic C-H out-of-plane bending

NMR (MeOD): (Fig 24)

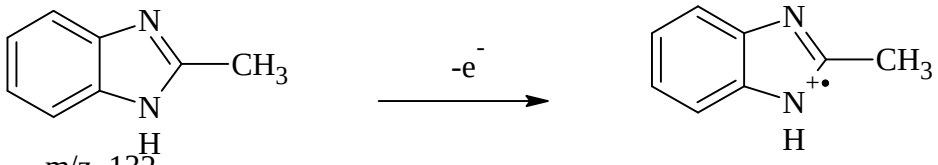


(4 aromatic protons, 3 aliphatic protons and one proton on nitrogen)

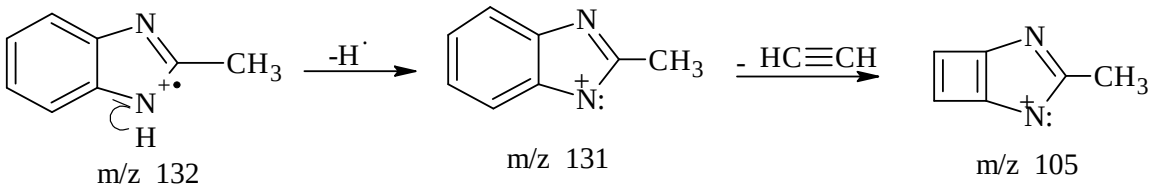
δ – Value	**Assignment**
7.70	(2H, m, Ar-H of C_4 and C_7)
7.26	(2H, m, Ar-H of C_5 and C_6)
5.0	(1H, s, broad, NH)
2.42	(3H, s, $-CH_3$)

MASS: (Fig 25)

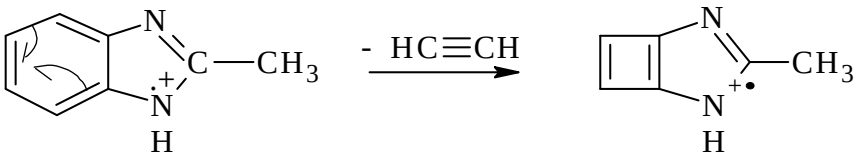
The structure of the compound was further confirmed by its fragmentation peaks which are as follows:



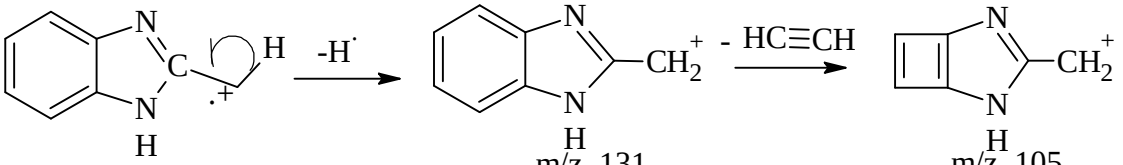
m/z 132 m/z 132 (M^+ peak)



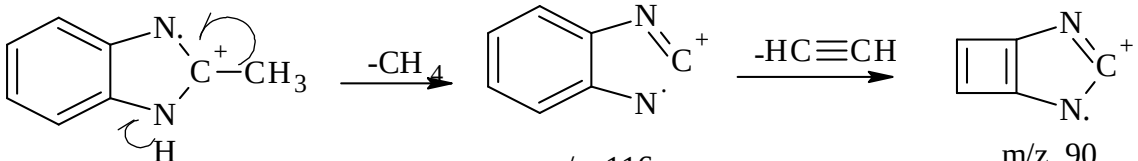
m/z 132 m/z 131 m/z 105



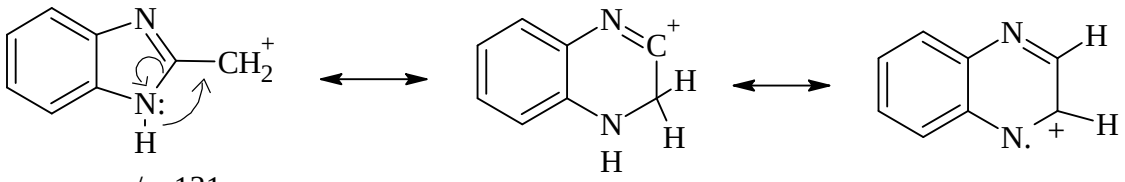
m/z 132 (base peak) m/z 106



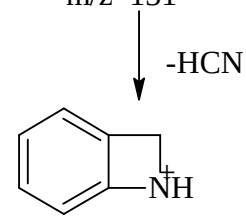
m/z 132 m/z 131 m/z 105



m/z 132 m/z 116 m/z 90



m/z 131 m/z 131 m/z 131



m/z 104

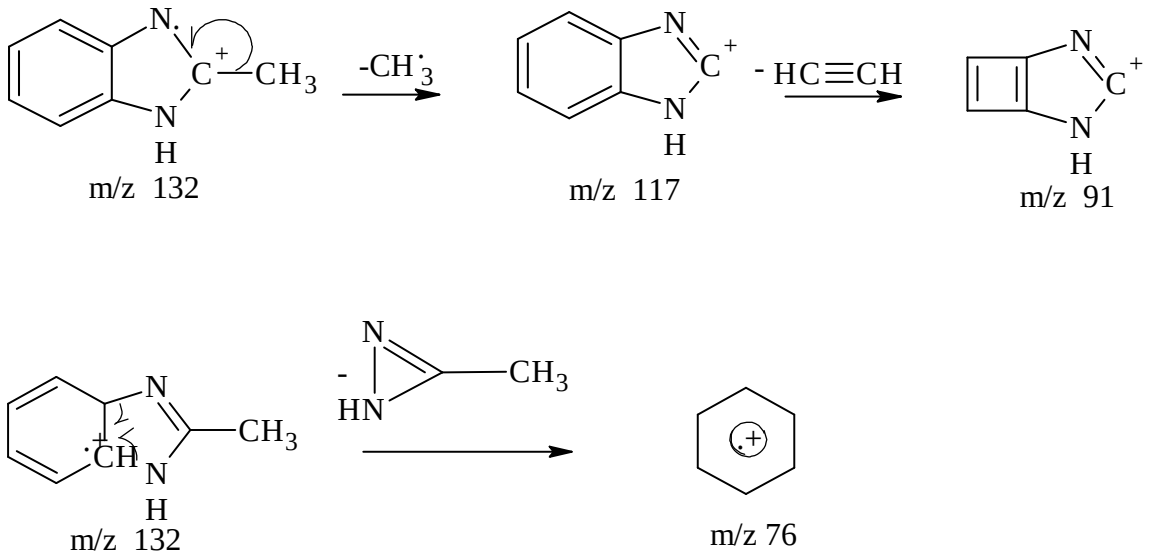


Fig 21: Fragmentation pattern of 2-methyl benzimidazole (SR_1)

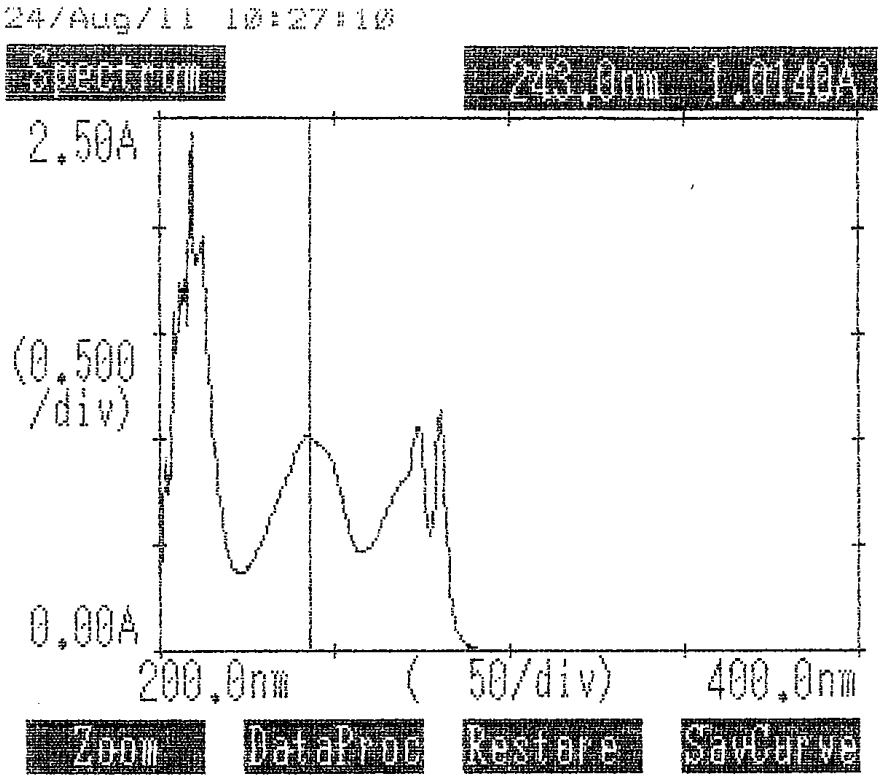


Fig 22: UV spectrum of the compound SR_1

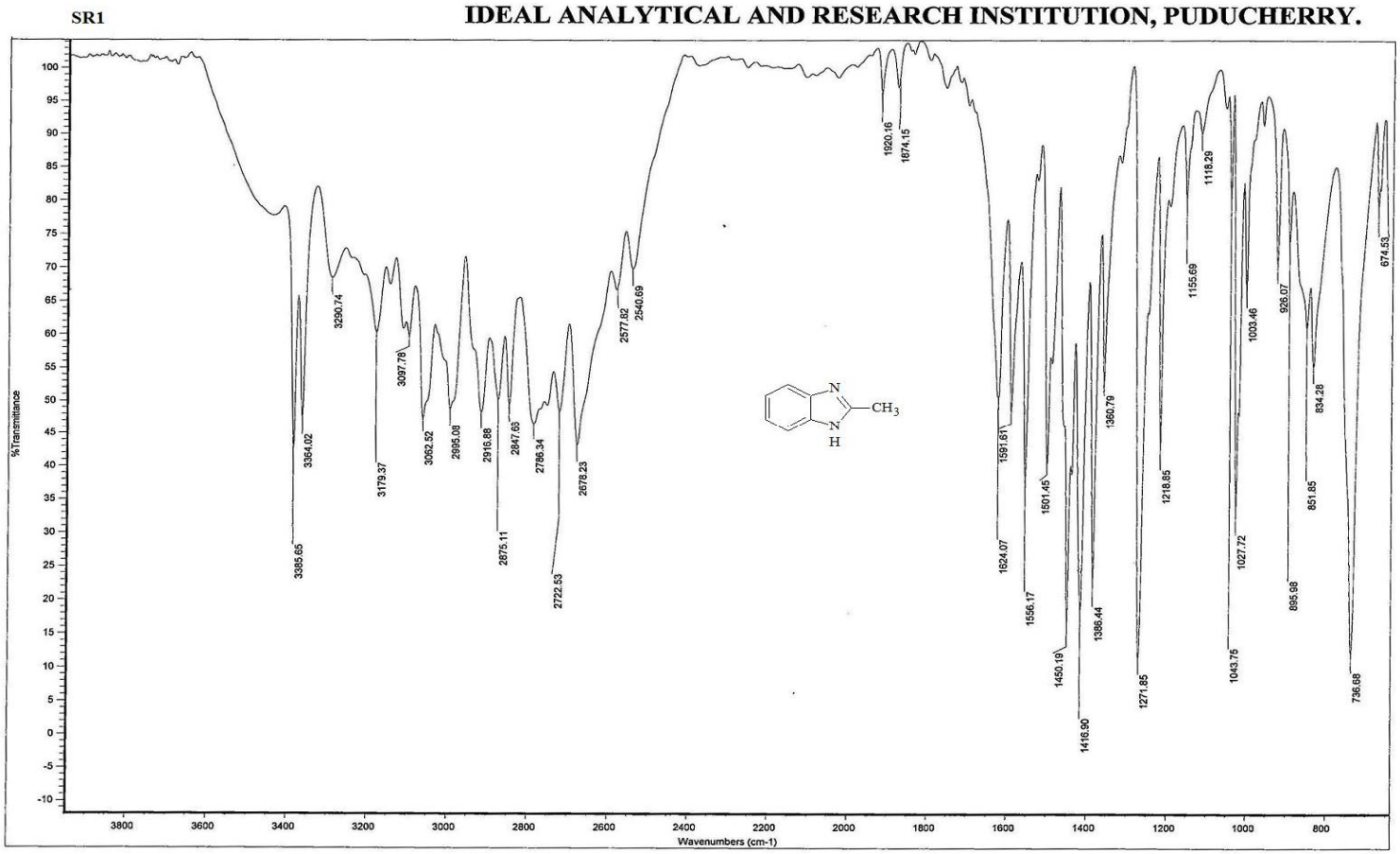
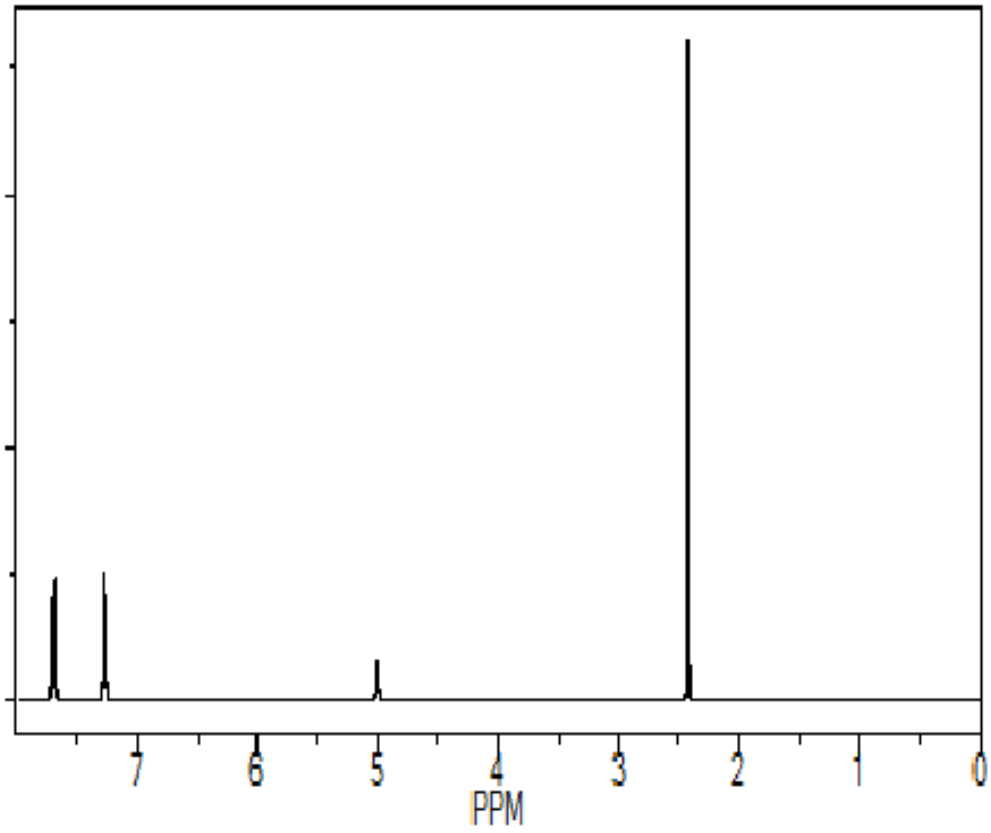


Fig 23: IR spectrum of the compound SR_1

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TD                 32768
SOLVENT             MeOD
NS                    32
DS                     2
SWH            10330.578 Hz
FIDRES         0.315264 Hz
AQ             1.5860212 sec
RG                   203
DW                48.400 usec
DE                 6.50 usec
TE                298.2 K
D1             1.00000000 sec
TD0                    1

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NUC1                  1H
P1                 10.65 usec
PL1                0.00 dB
PL1W        23.53637505 W
SFO1         500.1330885 MHz

F2 - Processing parameters
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SSB                    0
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GB                     0
PC                 1.00
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Fig 24: ^{1}H NMR spectrum of the compound SR_1

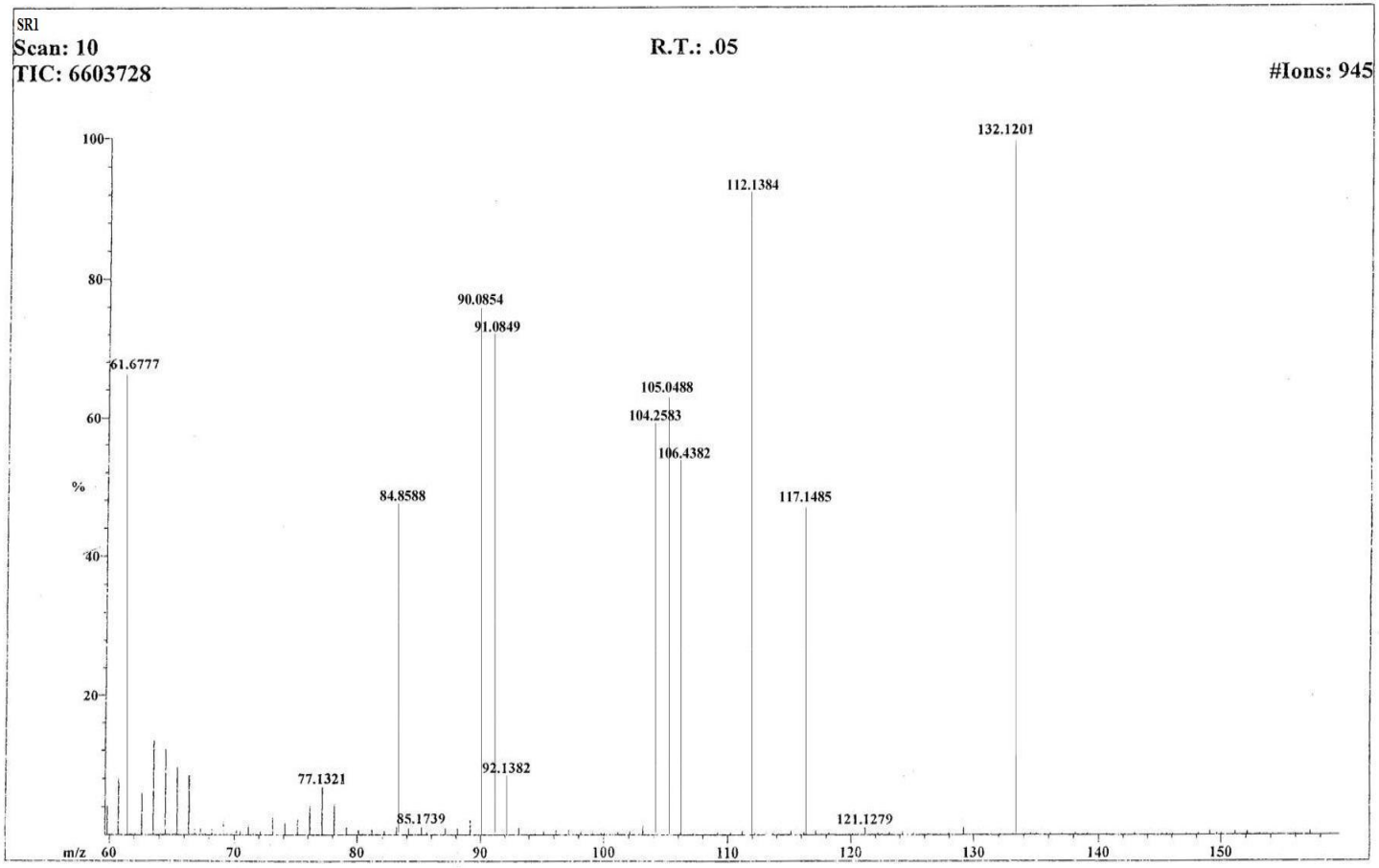


Fig 25: Mass spectrum of the compound SR_1

6.2.2 Spectral analysis of 2-ethyl benzimidazole (SR_2):

UV (MeOH): (Fig 27)

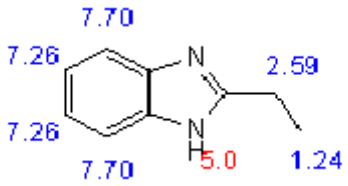
λ_{max} - 243.0 **(**ε_{max} 0.4058)

λ_{max} - 274.0 **(**ε_{max} 0.4591)

IR (KBr): (Fig 28)

Wave number (cm^{-1})	**Assignment**
3164 (s)	N-H stretching
3052 (w)	Aromatic (=C-H) stretching
2973 (m)	Aliphatic C-H stretching in $-CH_2$
1622 (s)	C=C stretching
1589 (s)	C=N stretching
1324 (s)	CH_3 bending
1457 (m)	CH_2 bending
794 (s)	Aromatic C-H out-of-plane bending

NMR (MeOD): (Fig 29)

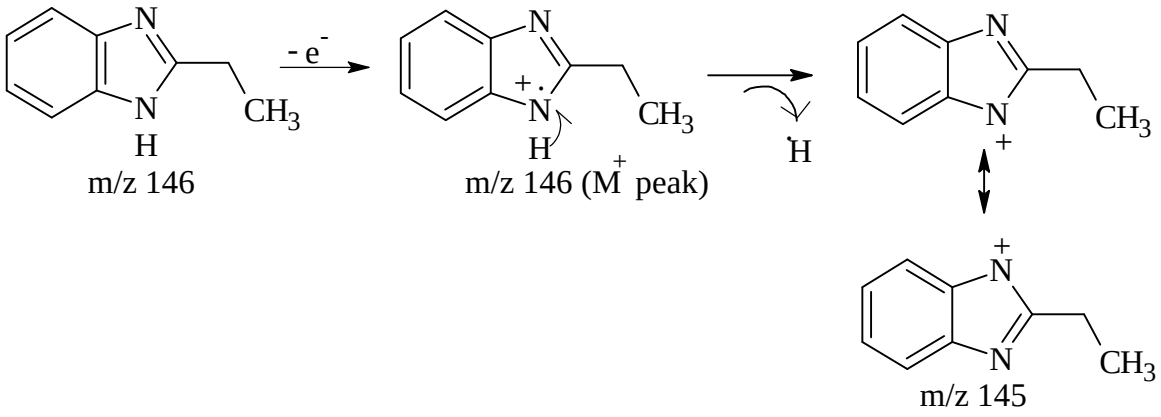


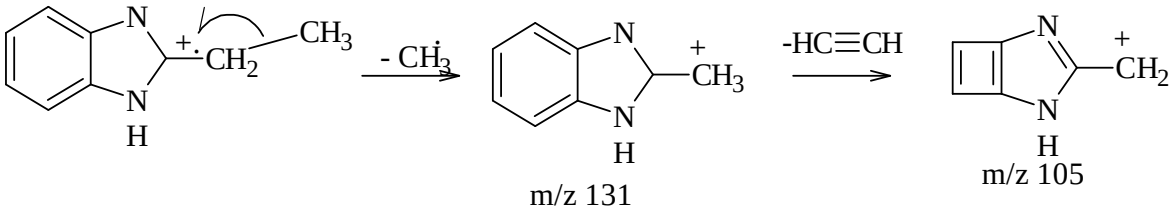
(4 aromatic protons, 5 aliphatic protons and one proton on nitrogen)

δ – Value	Assignment
7.70	(2H, m, Ar-H of C_4 andC_7)
7.26	(2H, m, Ar-H of C_5 and C_6)
5.0	(1H, s, broad, NH)
2.59	(2H, q, CH_2, broad)
1.24	(3H, t, -CH_3)

MASS: (Fig 30)

The structure of the compound was further confirmed by its fragmentation peaks which are as follows:





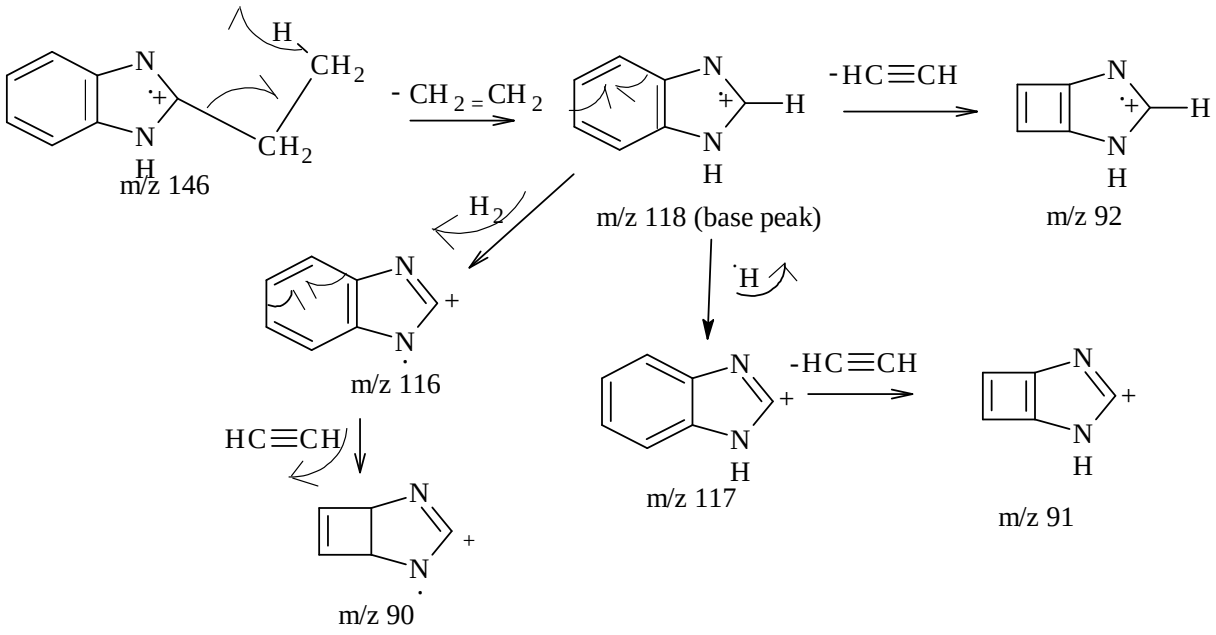


Fig 26: Fragmentation pattern of 2-ethyl benzimidazole (SR_2)

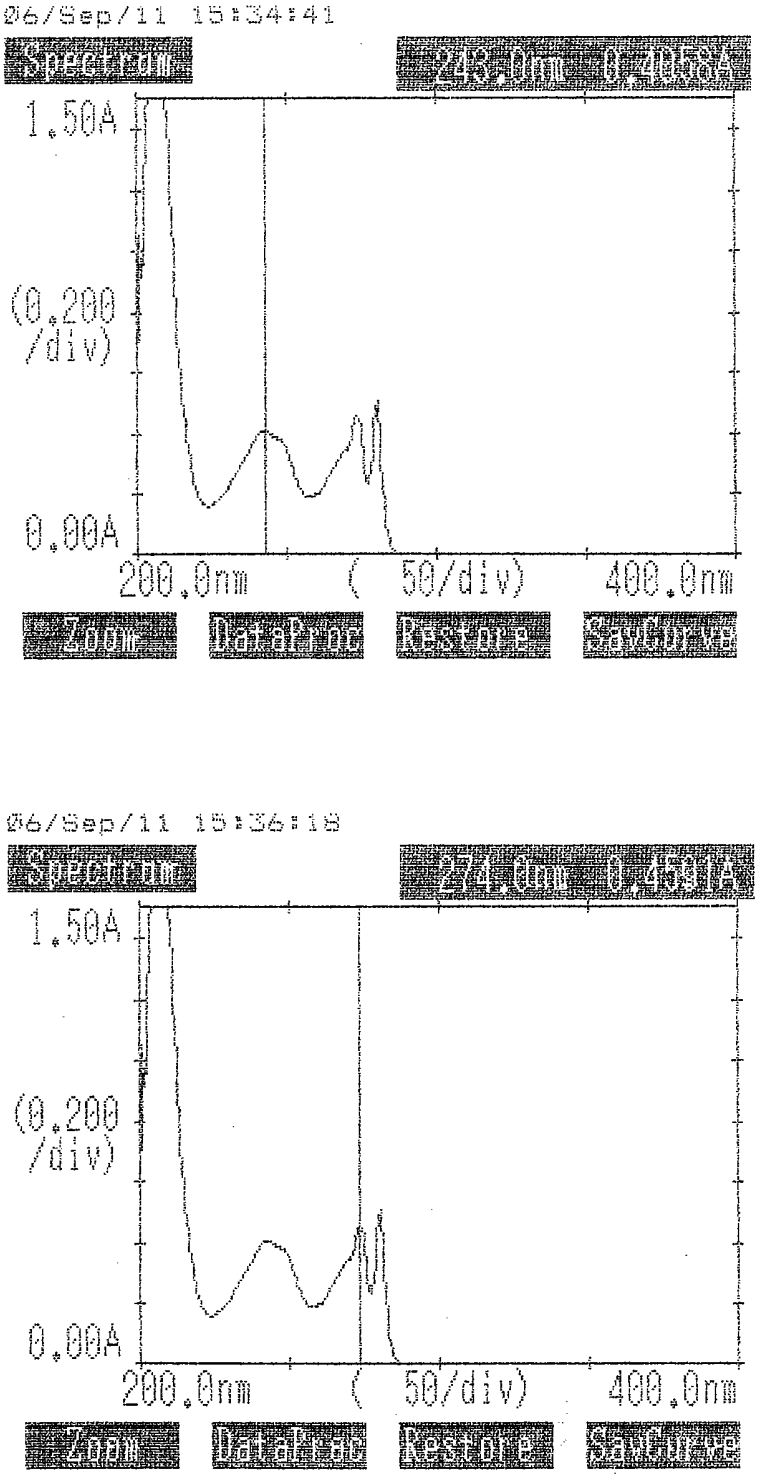


Fig 27: UV spectrum of compound SR_2

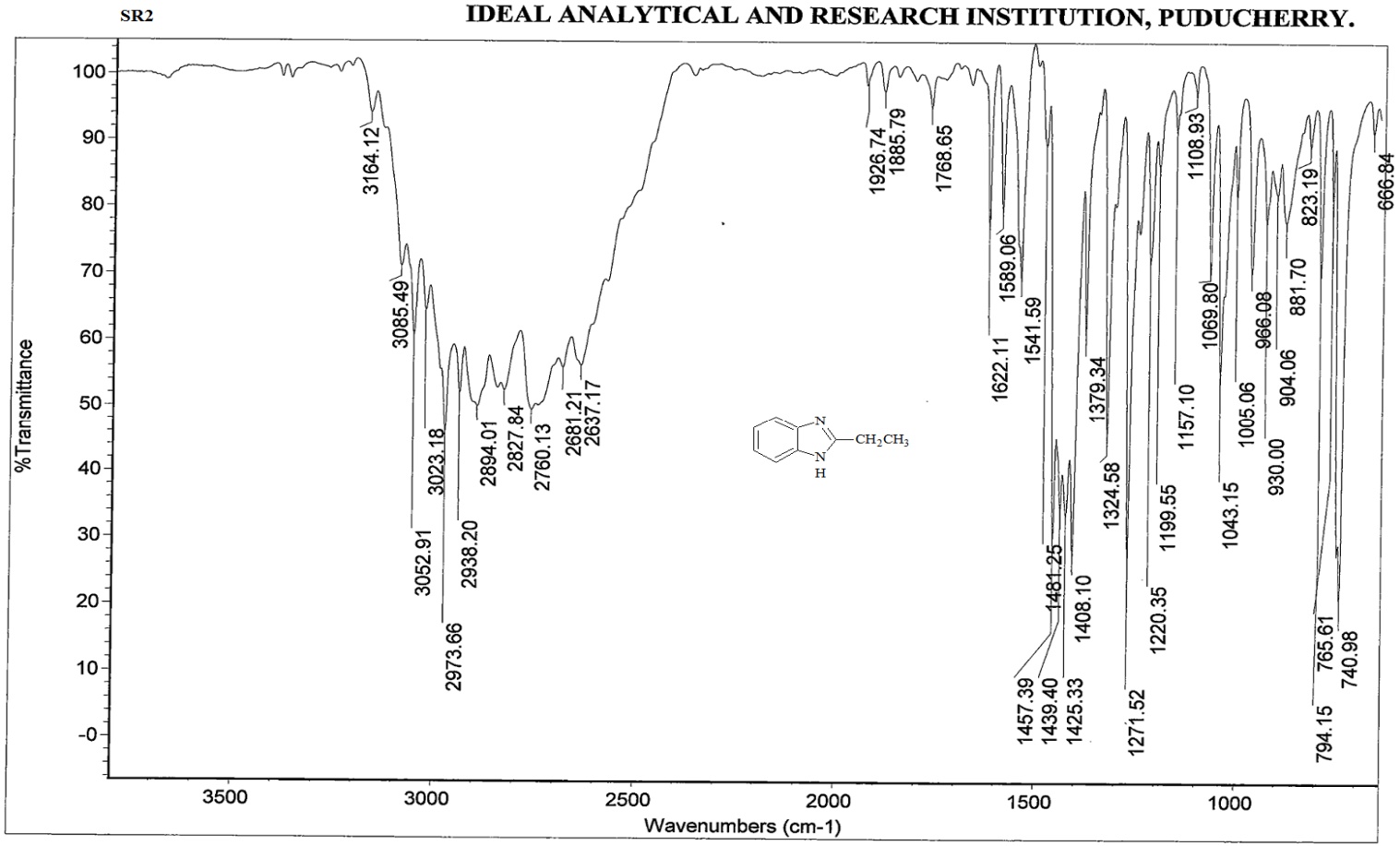
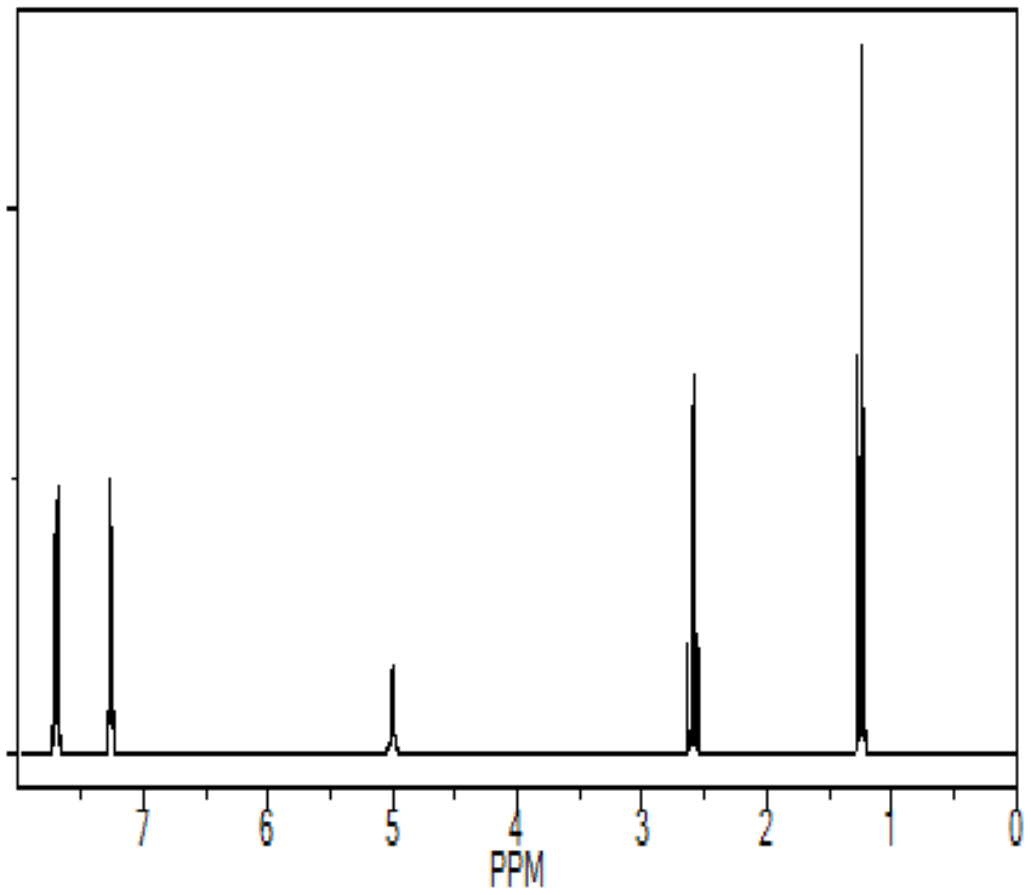


Fig 28: IR spectrum of compound SR_2

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TD                 32768
SOLVENT             MeOD
NS                    32
DS                     2
SWH            10330.578 Hz
FIDRES         0.315264 Hz
AQ             1.5860212 sec
RG                   203
DW                48.400 usec
DE                 6.50 usec
TE                298.2 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                10.65 usec
PL1                0.00 dB
PL1W        23.53637505 W
SFO1        500.1330885 MHz

F2 - Processing parameters
SI                 32768
SF          500.1300000 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                  1.00
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Fig 29: ^{1}H NMR spectrum of compound SR_2

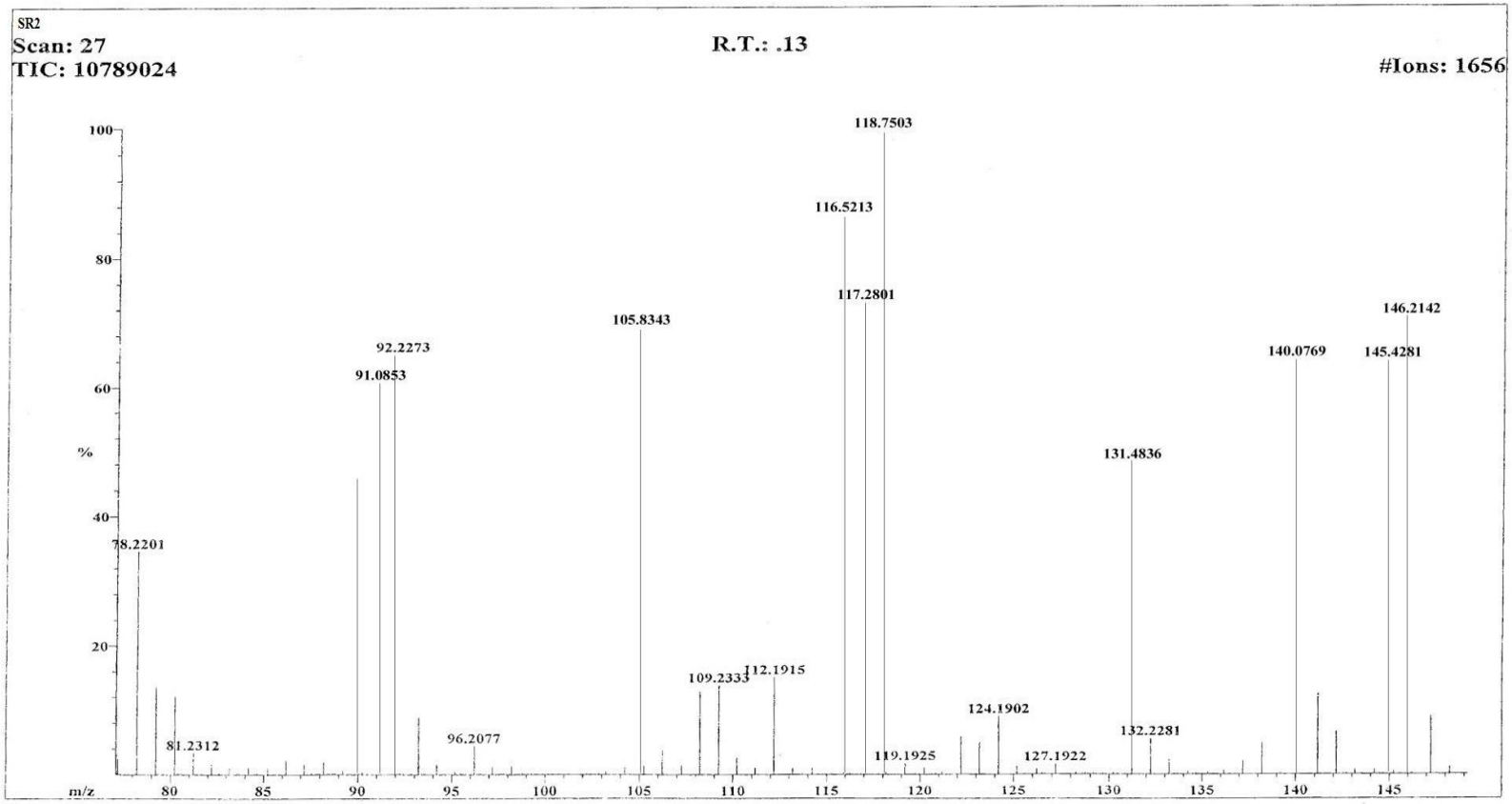


Fig 30: Mass spectrum of the compound SR_2

6.2.3 Spectral analysis of 2-propyl benzimidazole (SR_3):

UV (MeOH): (Fig 32)

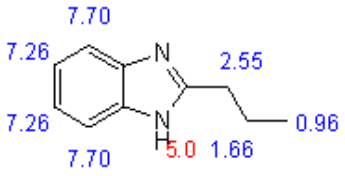
λ_{max} - 239.0 (ε_{max} 0.5980)

λ_{max} - 296.5 (ε_{max} 0.2899)

IR (KBr): (Fig 33)

Wave number (cm^{-1})	**Assignment**
3385 (s)	N-H stretching
3055 (w)	Aromatic (=C-H) stretching
2957 (m)	Aliphatic C-H stretching in $-CH_2$
1632 (s)	C=C stretching
1591 (s)	C=N stretching
1320 (s)	CH_3 bending
1457 (w)	CH_2 bending
748 (s)	Aromatic C-H bending

NMR (MeOD): (Fig 34)

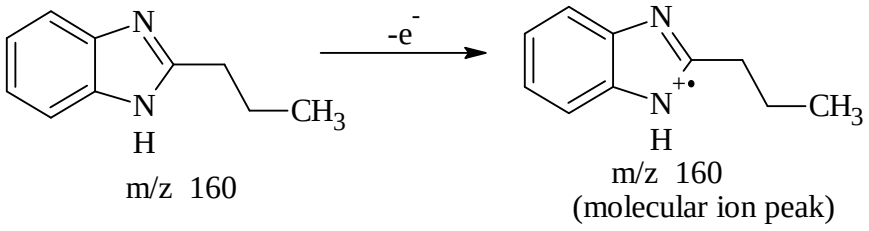


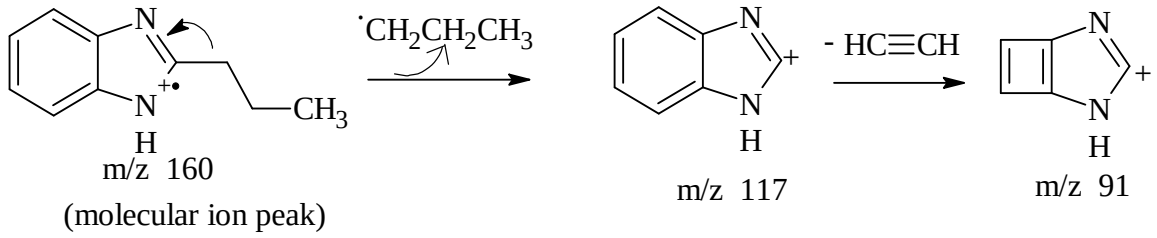
(4 aromatic protons, 7 aliphatic protons and one proton on nitrogen)

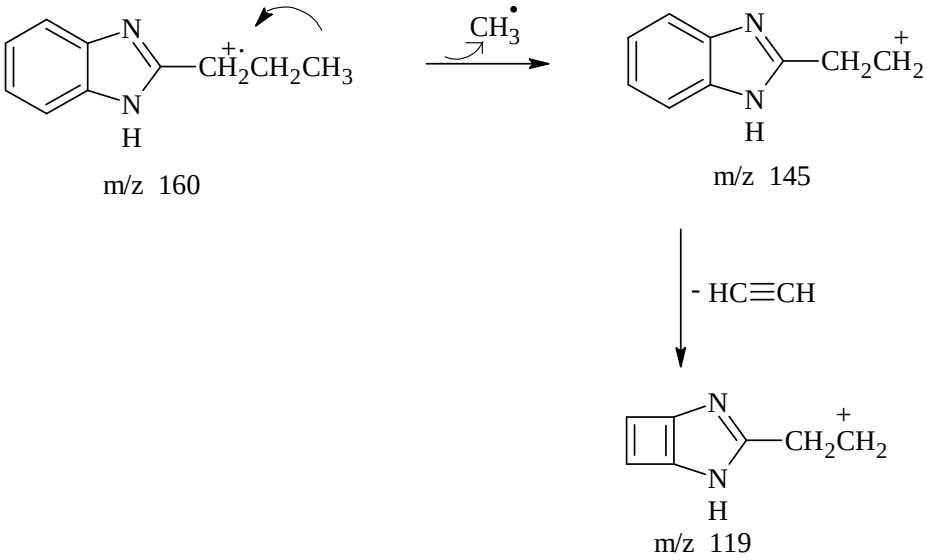
δ – Value	Assignment
7.70	(2H, m, Ar-H of C_4 and C_7)
7.26	(2H, m, Ar-H of C_5 and C_6)
5.0	(1H, s, broad, NH)
2.55	(2H, t, $-CH_2$)
1.66	(2H, q, $-CH_2$)
0.96	(3H, t, $-CH_3$)

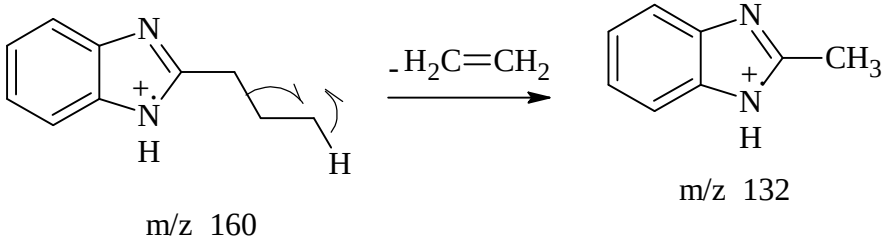
Mass: (Fig 35)

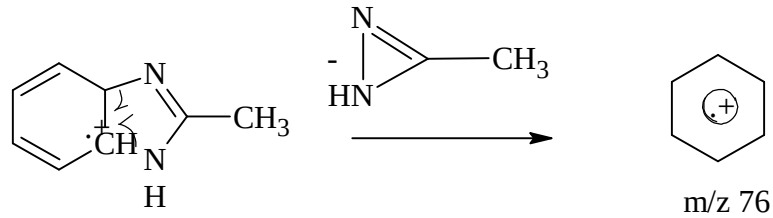
The structure of the compound was further confirmed by its fragmentation peaks which are as follows:

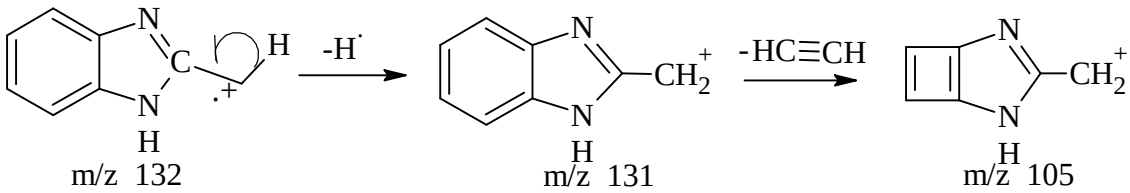


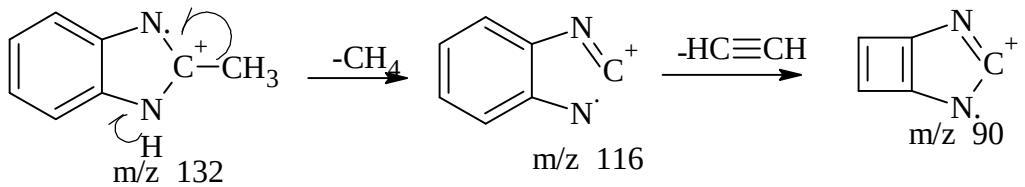



m/z 160
m/z 145
- HC≡CH
m/z 119


- H2C=CH2
m/z 160
m/z 132


m/z 132
m/z 76


-H·
-HC≡CH
m/z 132
m/z 131
m/z 105


-CH4
-HC≡CH
m/z 132
m/z 116
m/z 90

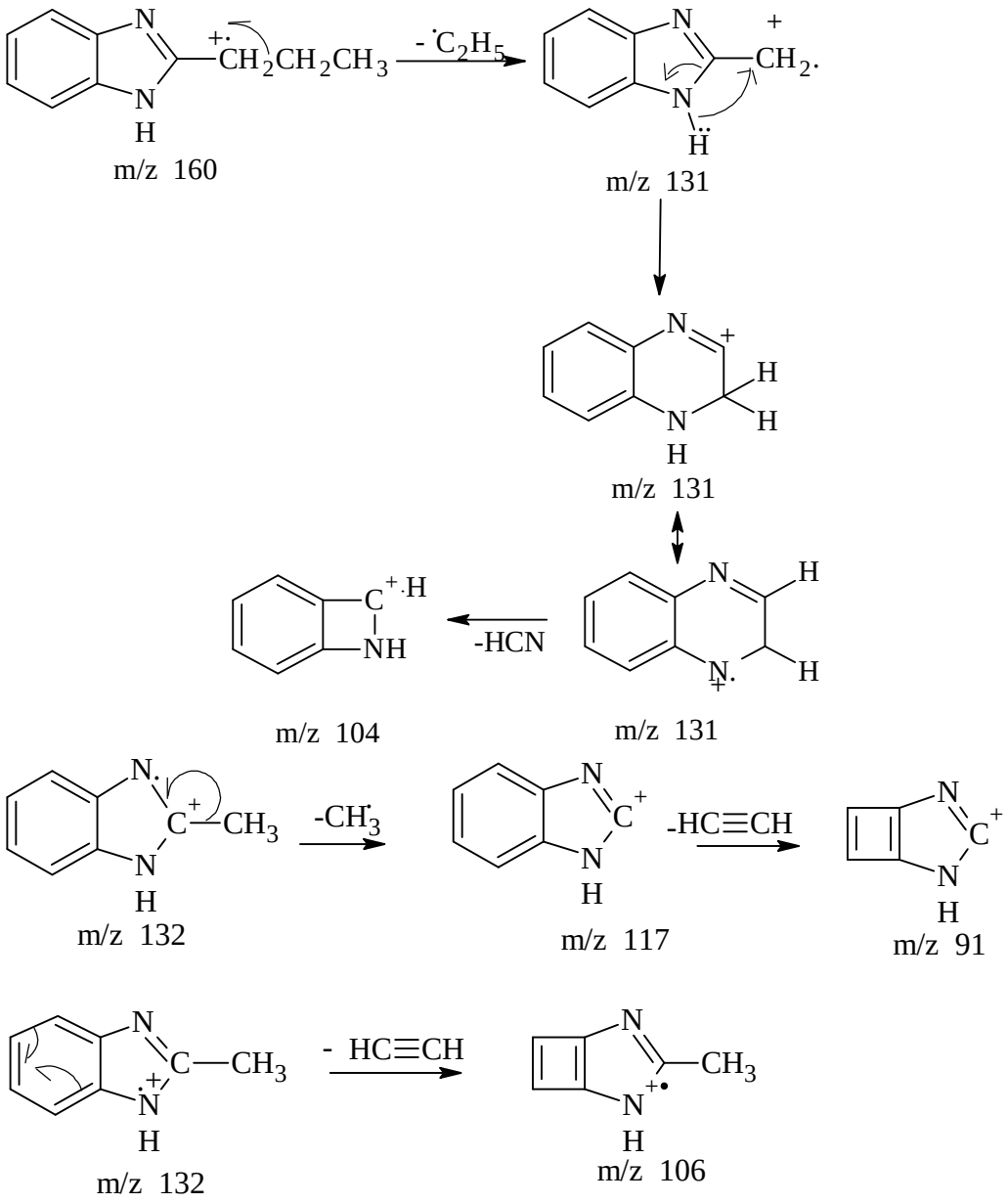


Fig 31: Fragmentation pattern of 2-propyl-benzimidazole (SR_3)

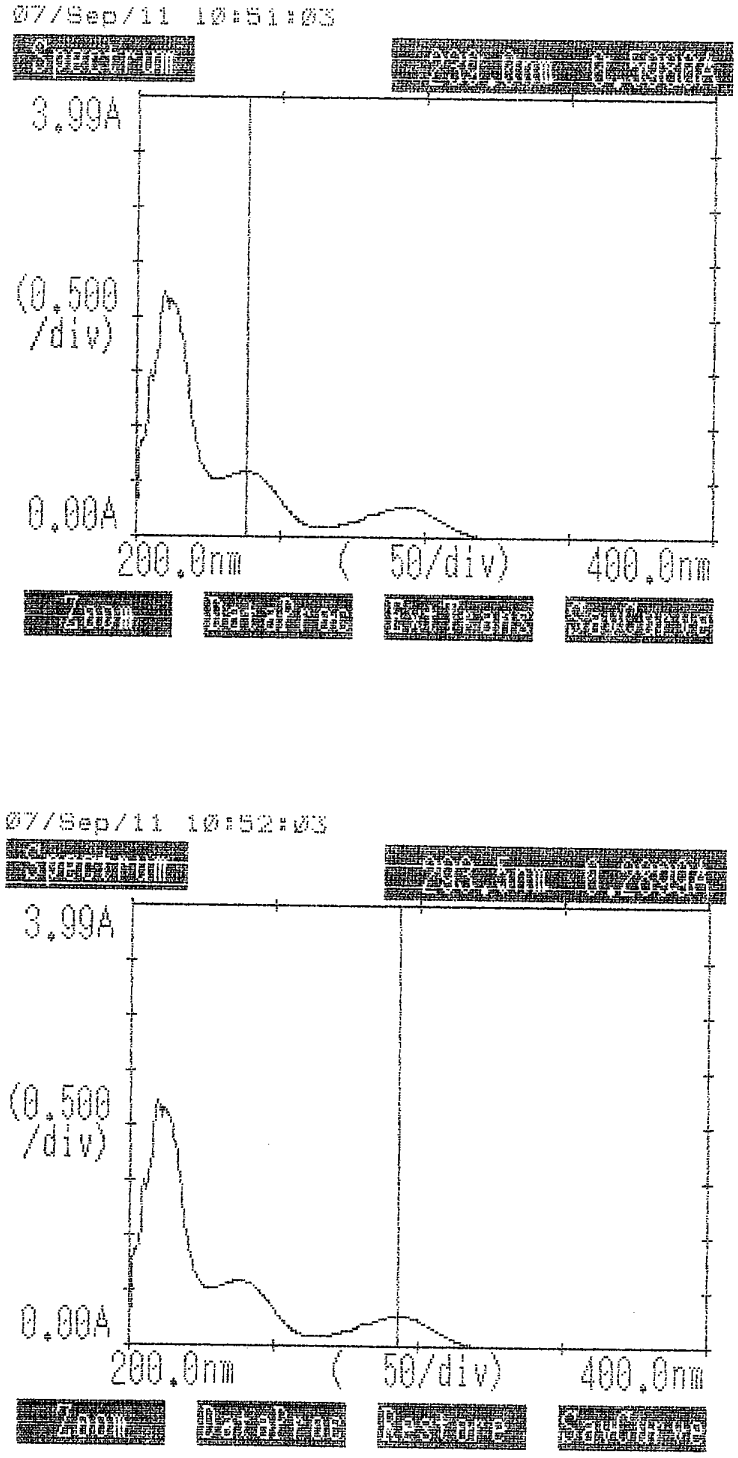


Fig 32: UV spectrum of compound SR_3

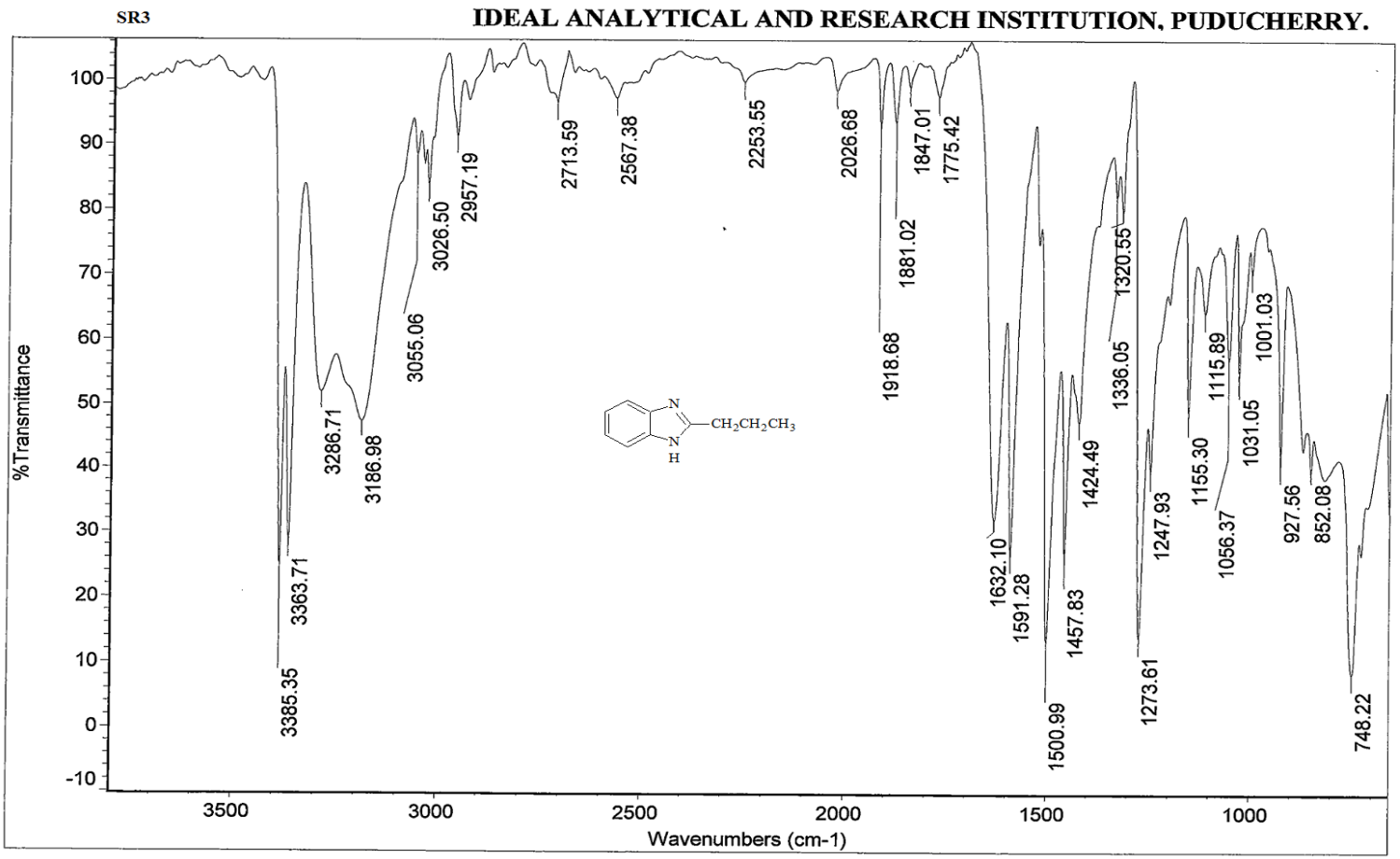
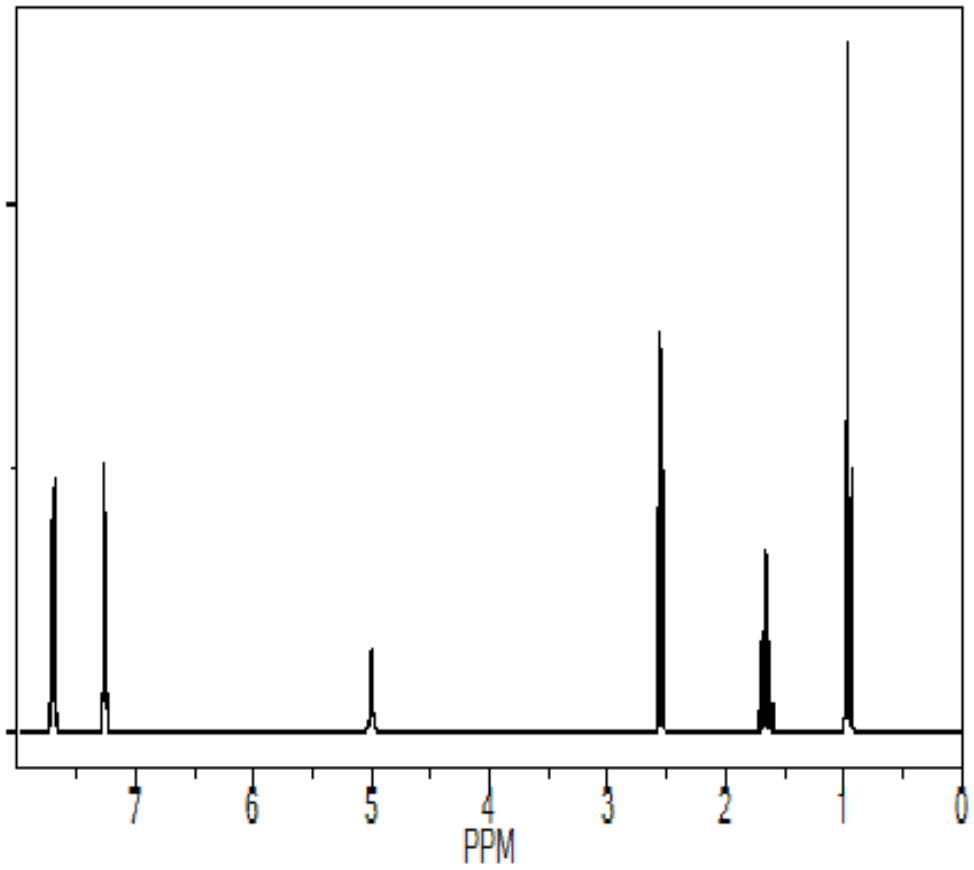


Fig 33: IR spectrum of compound SR_3

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PULPROG                   zg30
TD                       32768
SOLVENT                   MeOD
NS                          32
DS                           2
SWH                  10330.578 Hz
FIDRES                0.315264 Hz
AQ                    1.5860212 sec
RG                         203
DW                      48.400 usec
DE                       6.50 usec
TE                      298.2 K
D1                   1.00000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                        1H
P1                       10.65 usec
PL1                       0.00 dB
PL1W                23.53637505 W
SFO1                500.1330885 MHz

F2 - Processing parameters
SI                       32768
SF                  500.1300000 MHz
WDW                        EM
SSB                         0
LB                       0.30 Hz
GB                          0
PC                       1.00
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Fig 34: ^{1}H NMR spectrum of compound SR_3

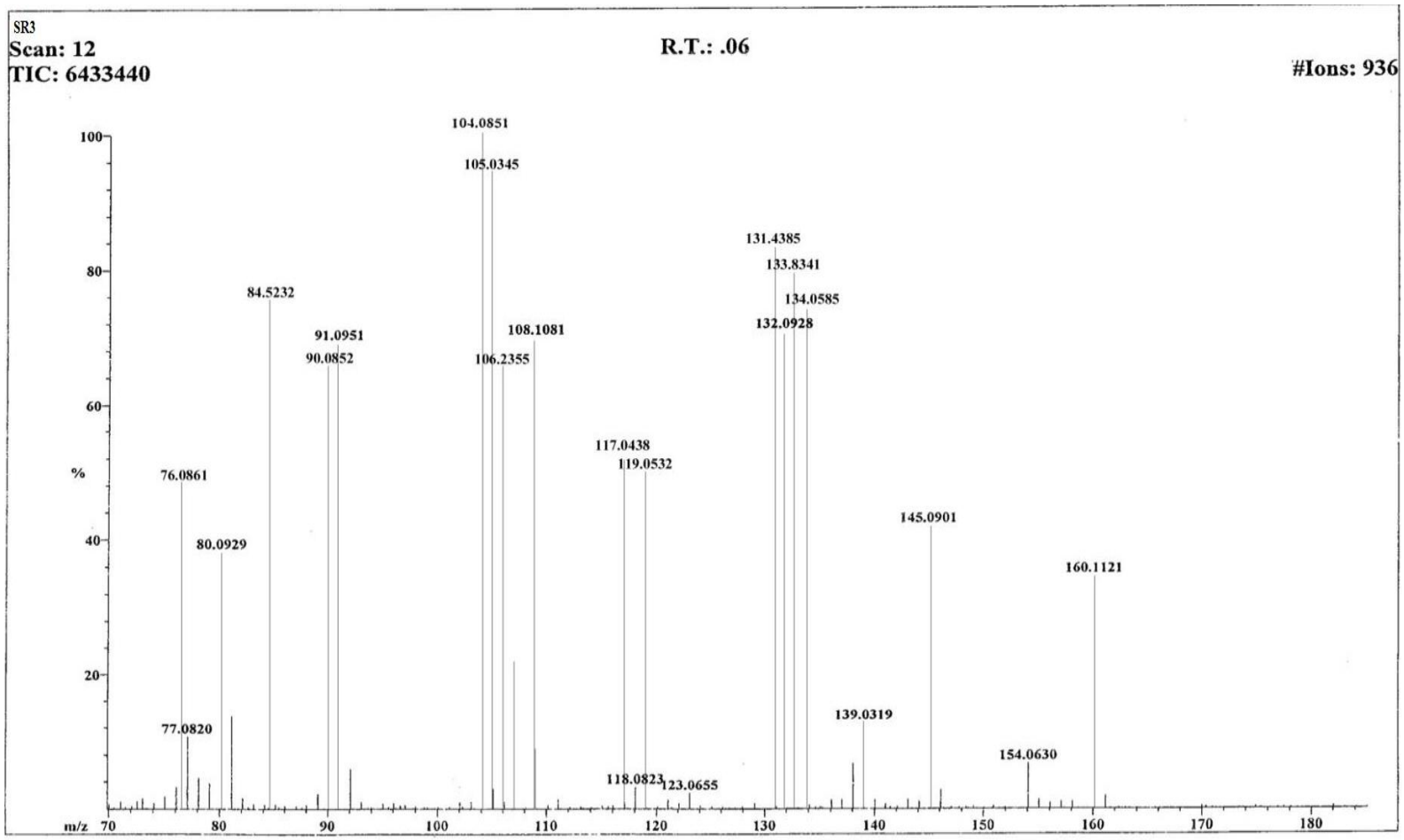


Fig 35: Mass spectrum of compound SR_3

6.2.4 Spectral analysis of 1-((sulphonomido)-methyl)-2-methyl benzimidazole (SR_4):

Spectroscopy Data:

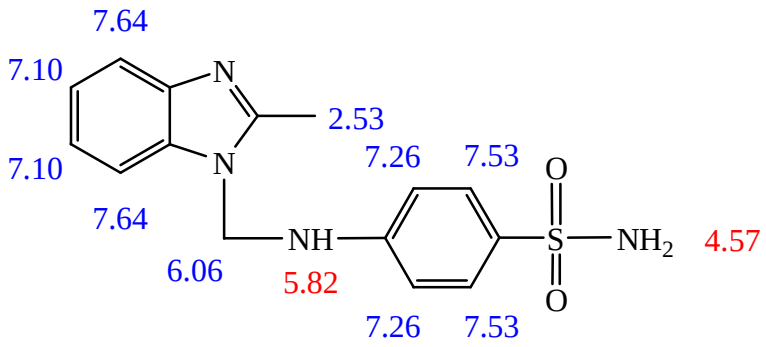
UV (MeOH): (Fig 37)

λ_{max} - 262.5 nm (ε_{max} – 1.4568)

IR (KBr): (Fig 38)

Wave number (cm^{-1})	**Assignment**
3376 (s)	1° N-H stretching in sulphonamide
3263 (s)	2° N-H stretching in sulphonamide
3063 (w)	Aromatic (=C-H) stretching
2921 (m)	C-H stretching in $–CH_2$
1595 (s)	C=N stretching
1460 (s)	C=C stretching
1438 (m)	CH_3 asymmetric bending
1312 (s)	SO_2 stretching
1386 (s)	C-N stretching (Aromatic tertiary amine)
746 (s)	C-H out-of-planes bending (Aromatic C-H)

NMR (DMSO-*d6*): (Fig 39)

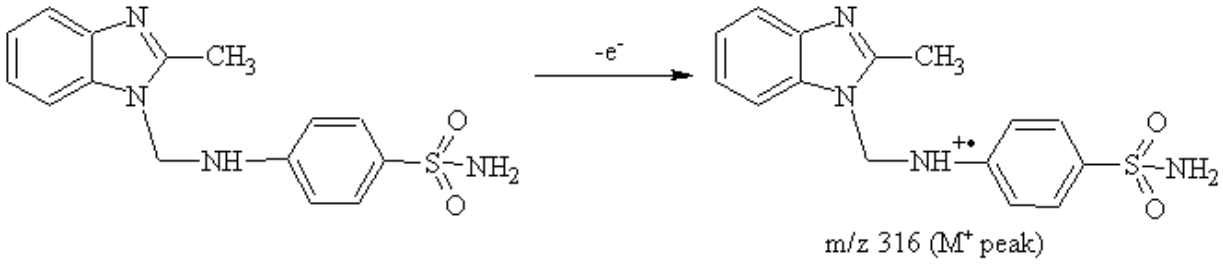


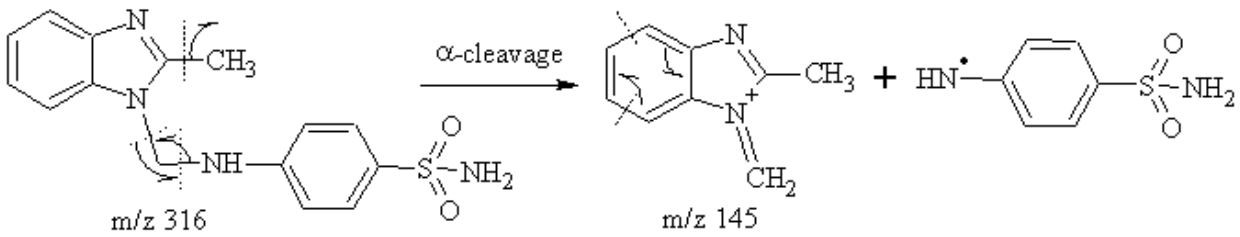
(8 aromatic protons, 5 aliphatic protons and 3 protons on nitrogen)

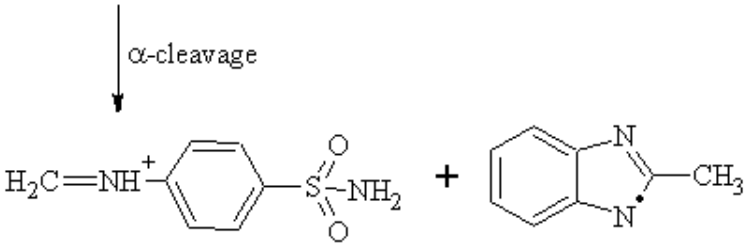
δ – Value	**Assignment**
7.10 – 7.64	(8H, m, Ar-H – C_4, C_5, C_6, C_7 and 4-phenyl protons)
6.06	(2H, s, $-CH_2$)
4.57	(2H, s, $-SO_2NH_2$)
5.82	(1H, s, -NH)
2.53	(3H, s, $-CH_3$)

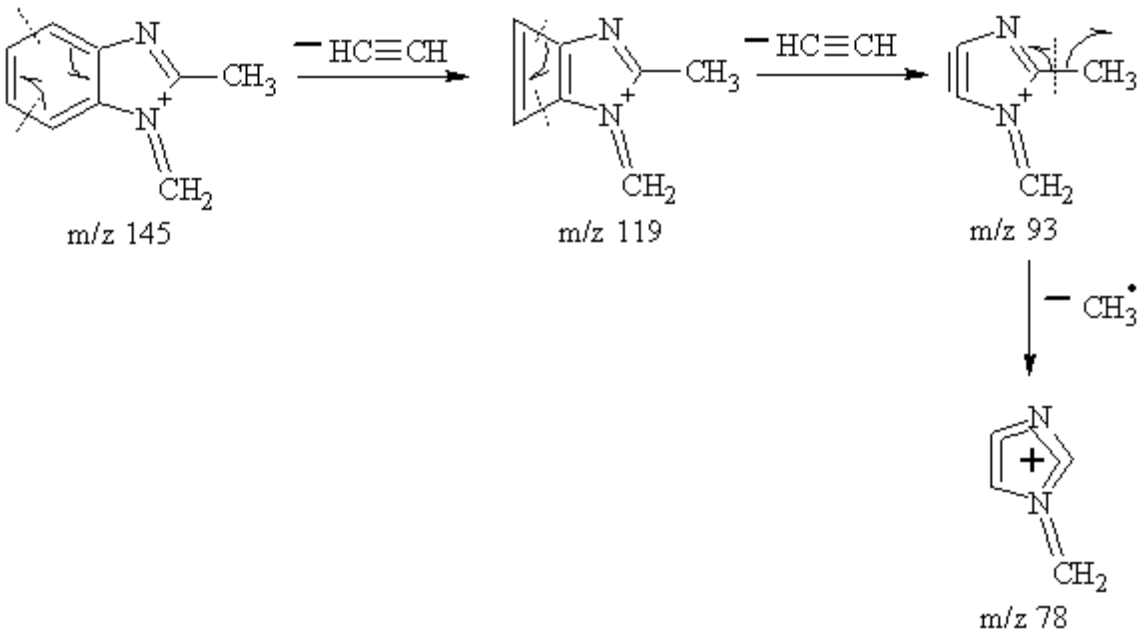
Mass: (Fig 40)

The structure of the compound was confirmed by its fragmentation peaks which are as follows:




α-cleavage
m/z 316
m/z 145


α-cleavage
m/z 185


− HC≡CH
− HC≡CH
m/z 145
m/z 119
m/z 93
− CH3•
m/z 78

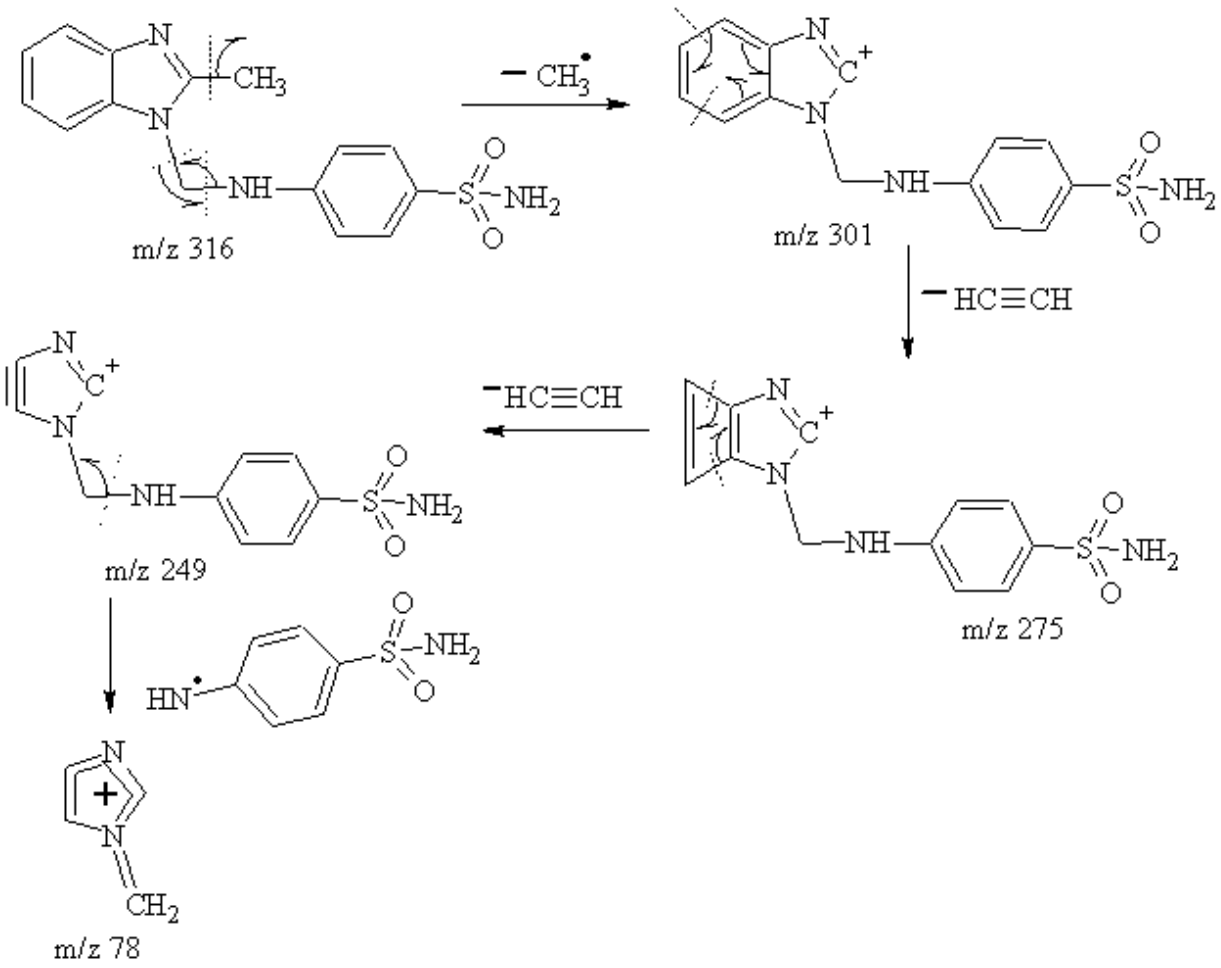


Fig 36: Fragmentation pattern of SR_4

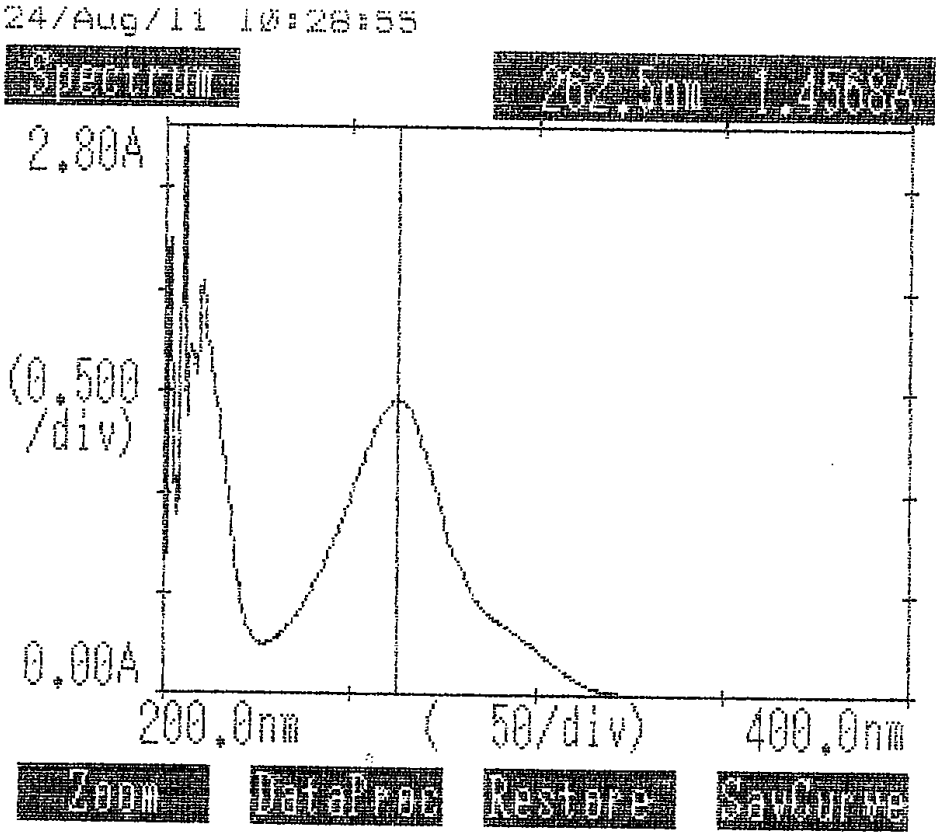


Fig 37: UV spectrum of the compound SR_4

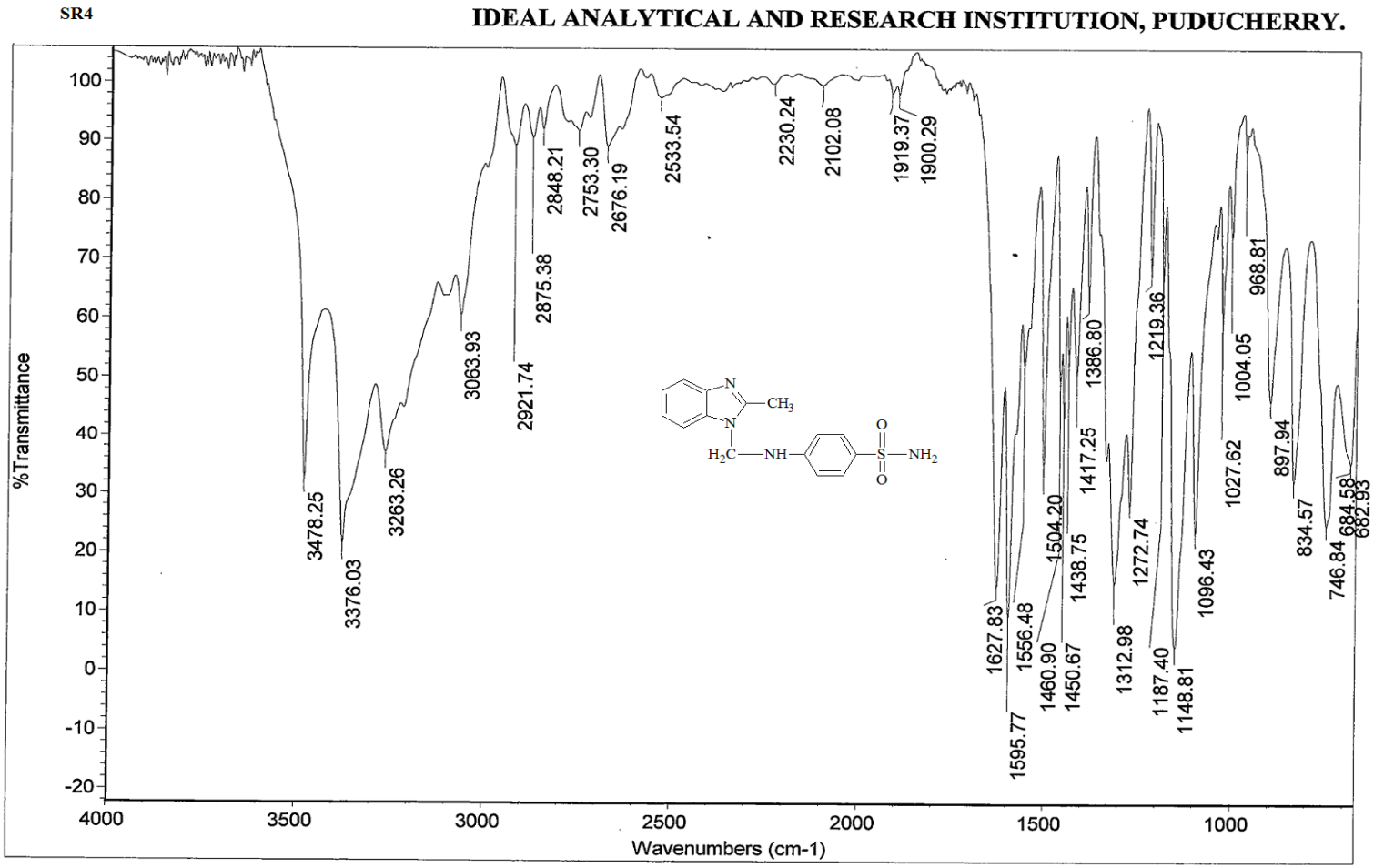


Fig 38: IR spectrum of the compound SR_4

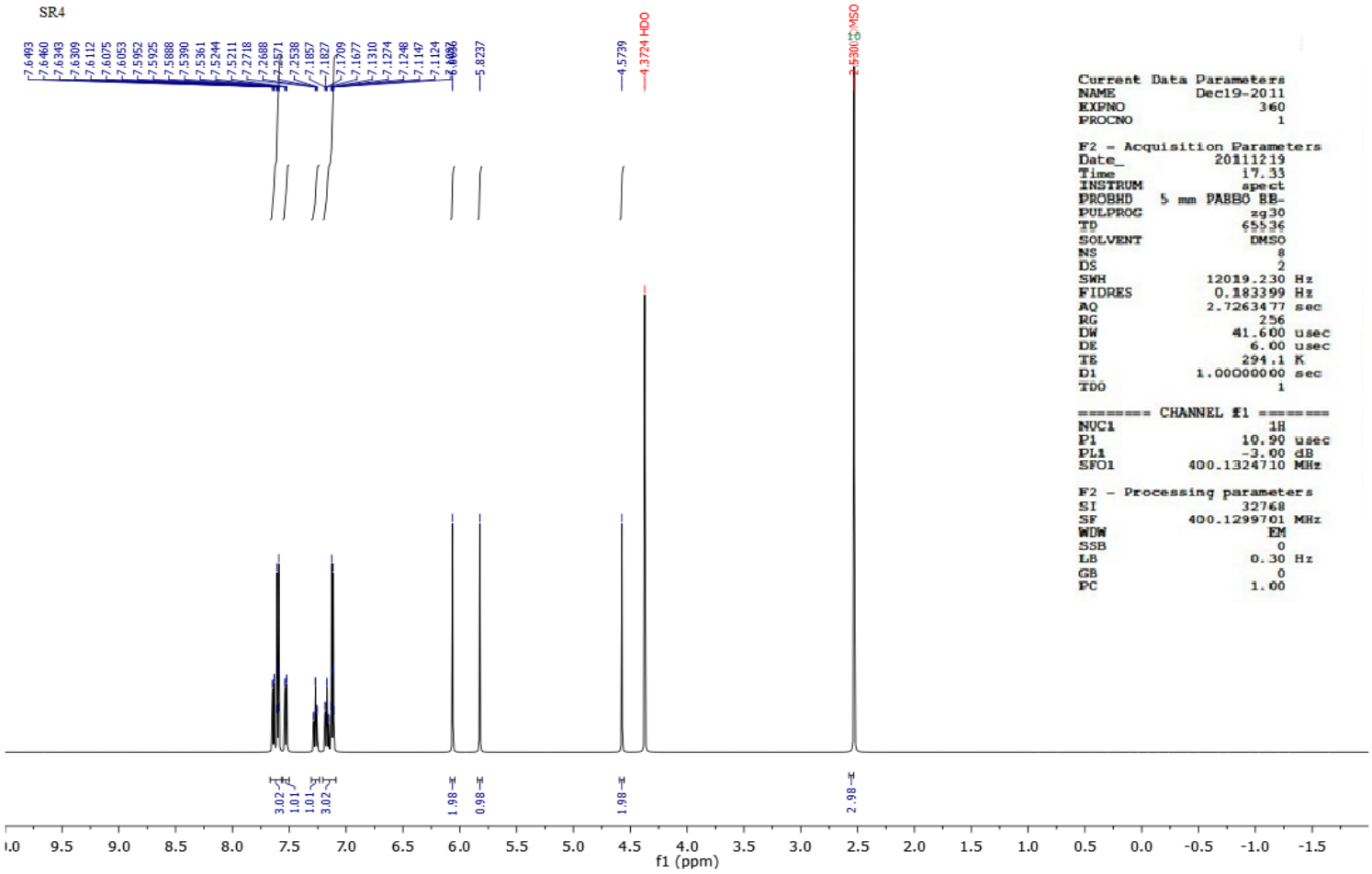


Fig 39: ^{1}H NMR spectrum of the compound SR_4

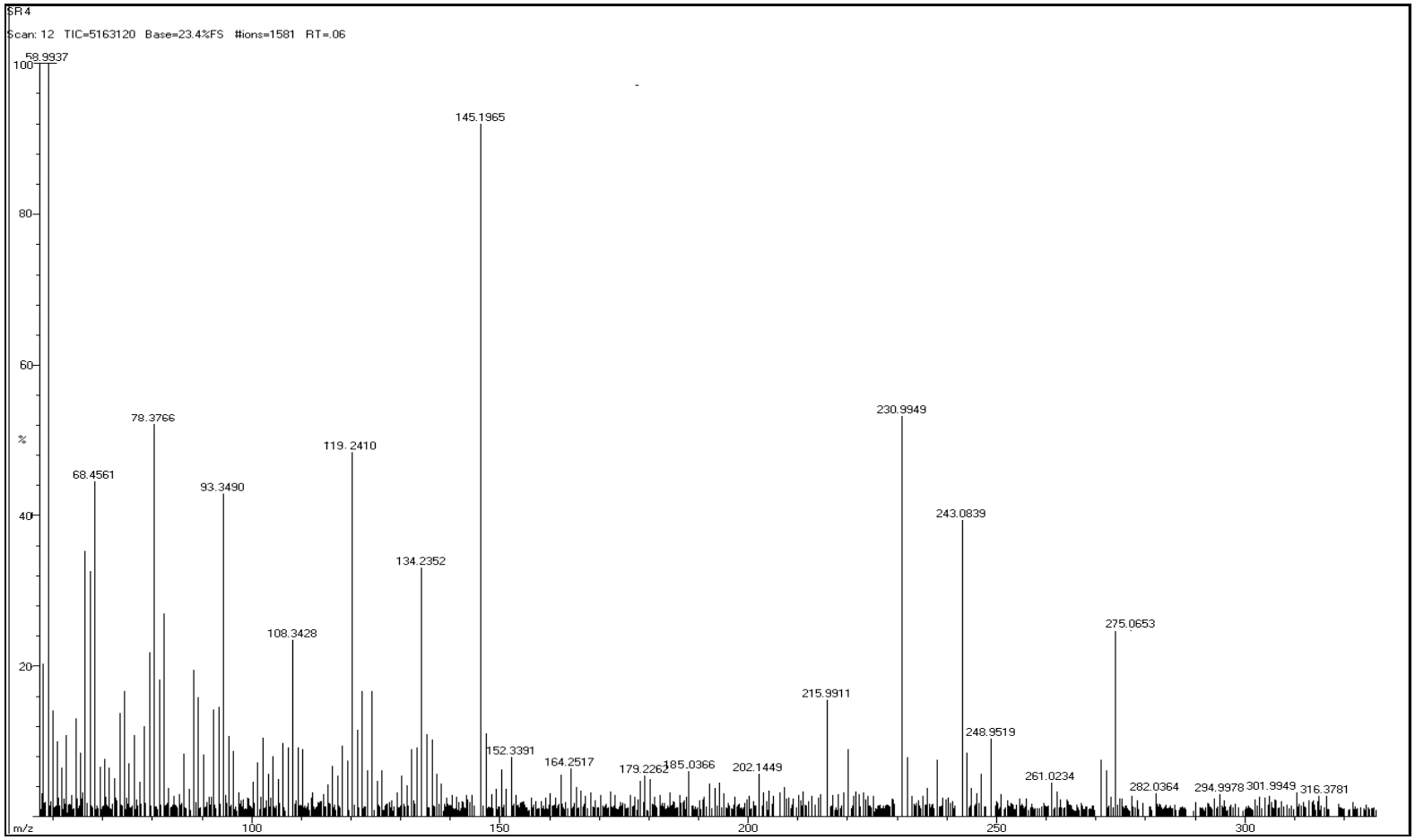


Fig 40: Mass spectrum of the compound SR_4

6.2.5 Spectral analysis of 1-((sulphonomido)-methyl)-2-ethyl benzimidazole (SR_5):

UV (MeOH): (Fig 42)

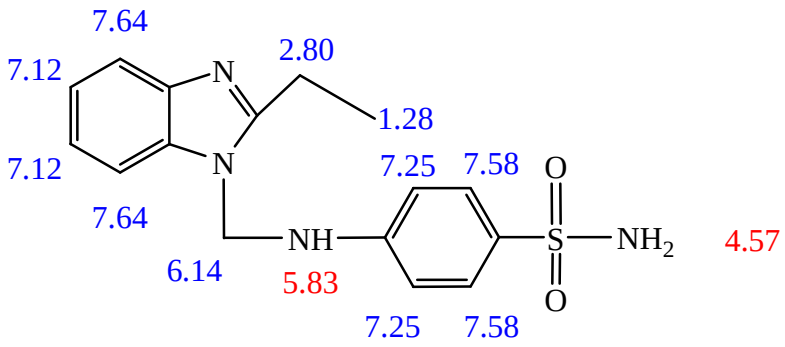
λ_{max} - 240.0 nm (ε_{max} – 0.7504)

λ_{max} - 293.0 nm (ε_{max} – 0.3855)

IR (KBr): (Fig 43)

Wave number (cm^{-1})	**Assignment**
3378 (s)	1° N-H stretching in sulphonamide
3263 (s)	2° N-H stretching in sulphonamide
3057 (w)	Aromatic (=C-H) stretching
2938 (m)	C-H stretching in $–CH_2$
1597 (s)	C=N stretching
1460 (s)	C=C stretching
1438 (m)	Aliphatic CH_2 bending
1313 (s)	SO_2 stretching
1387 (s)	C-N stretching (Aromatic tertiary amine)
744 (s)	C-H out-of-planes bending (Aromatic C-H)

NMR (DMSO-*d6*): (Fig 44)

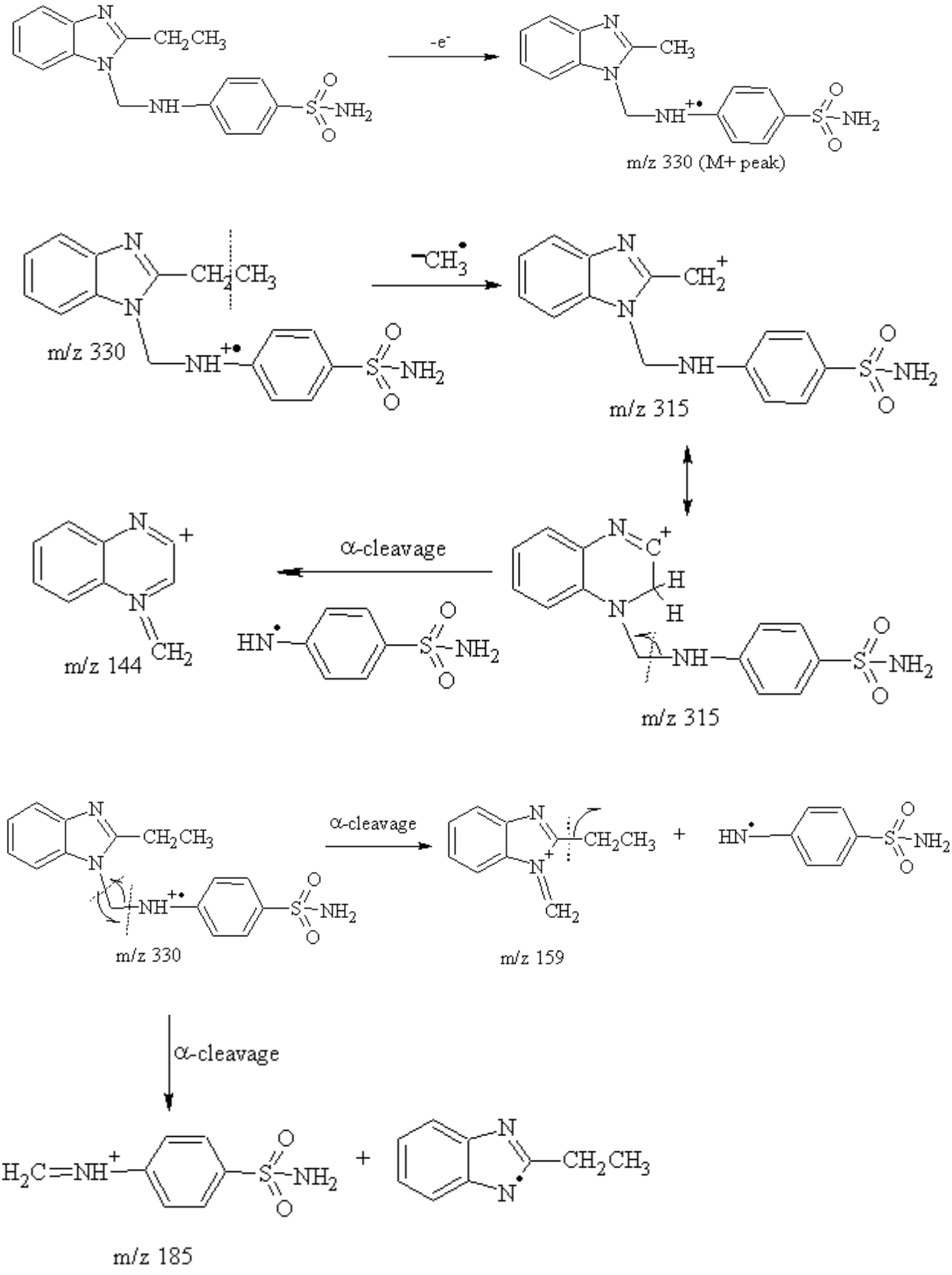


(8 aromatic protons, 7 aliphatic protons and 3 protons on nitrogen)

δ – Value	**Assignment**
7.12 – 7.64	(8H, m, Ar-H – C_4, C_5, C_6, C_7 and 4-phenyl protons)
6.14	(2H, s, $-CH_2$)
5.83	(1H, s, -NH)
4.57	(2H, s, $-SO_2NH_2$)
2.80	(2H, q, $-CH_2$)
1.28	(3H, t, $-CH_3$)

Mass: (Fig 45)

The structure of the compound was confirmed by its fragmentation peaks which are as follows:


-e⁻
m/z 330 (M+ peak)
m/z 330
m/z 315
m/z 315
α-cleavage
m/z 144
α-cleavage
m/z 330
m/z 159
α-cleavage
m/z 185

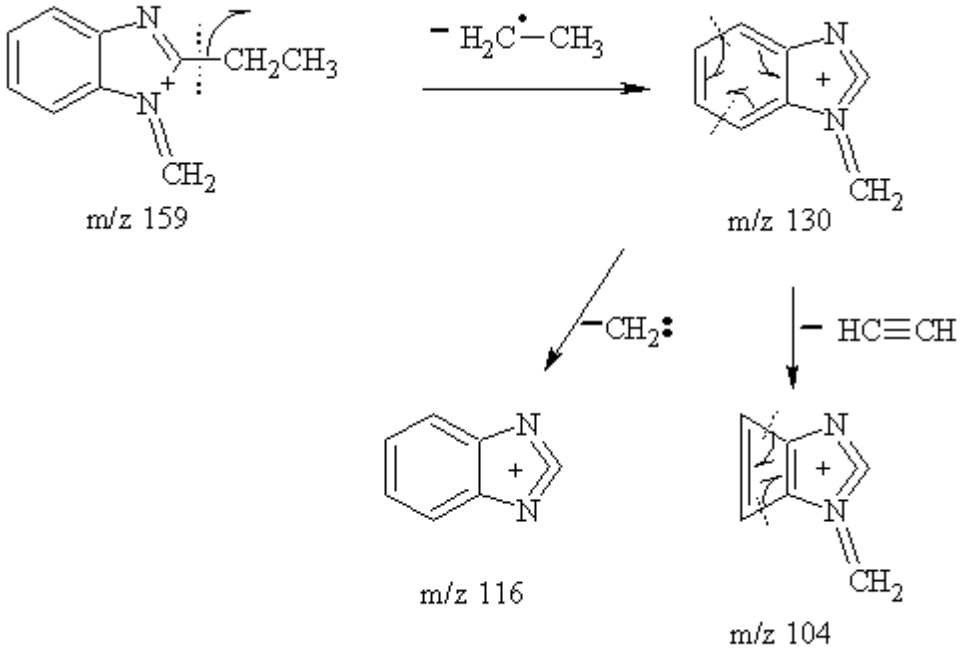
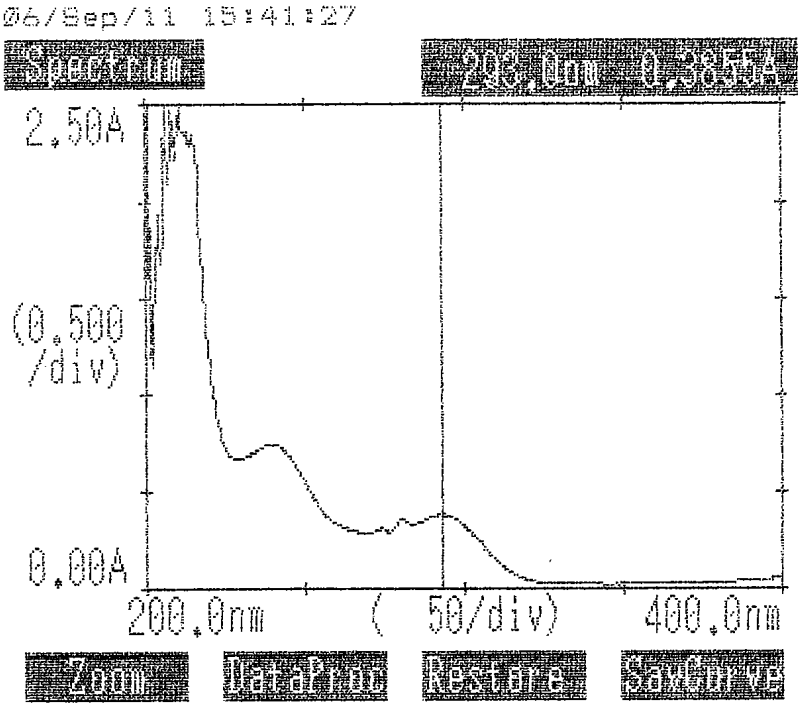


Fig 41: Fragmentation pattern of SR_5



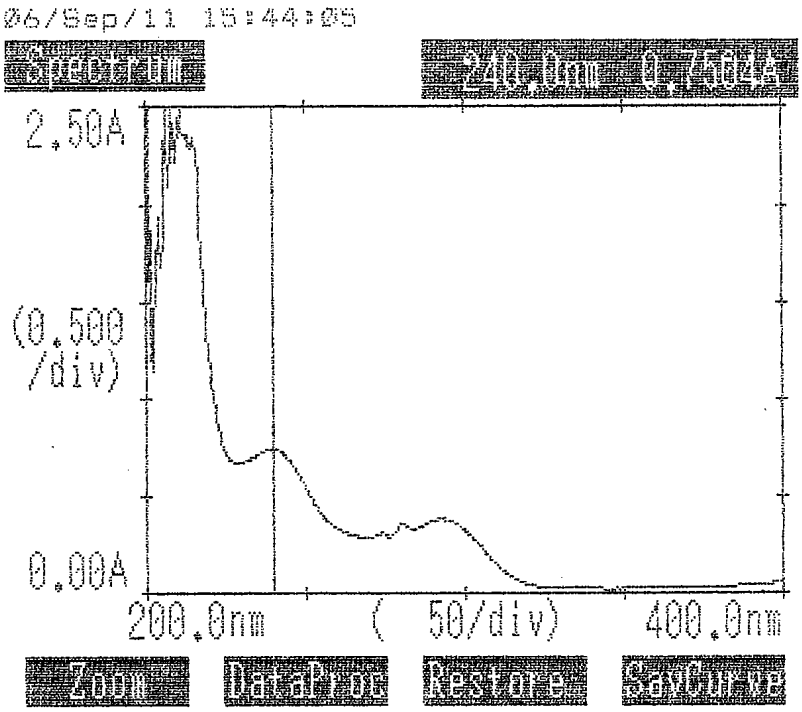


Fig 42: UV spectrum of the compound SR_5

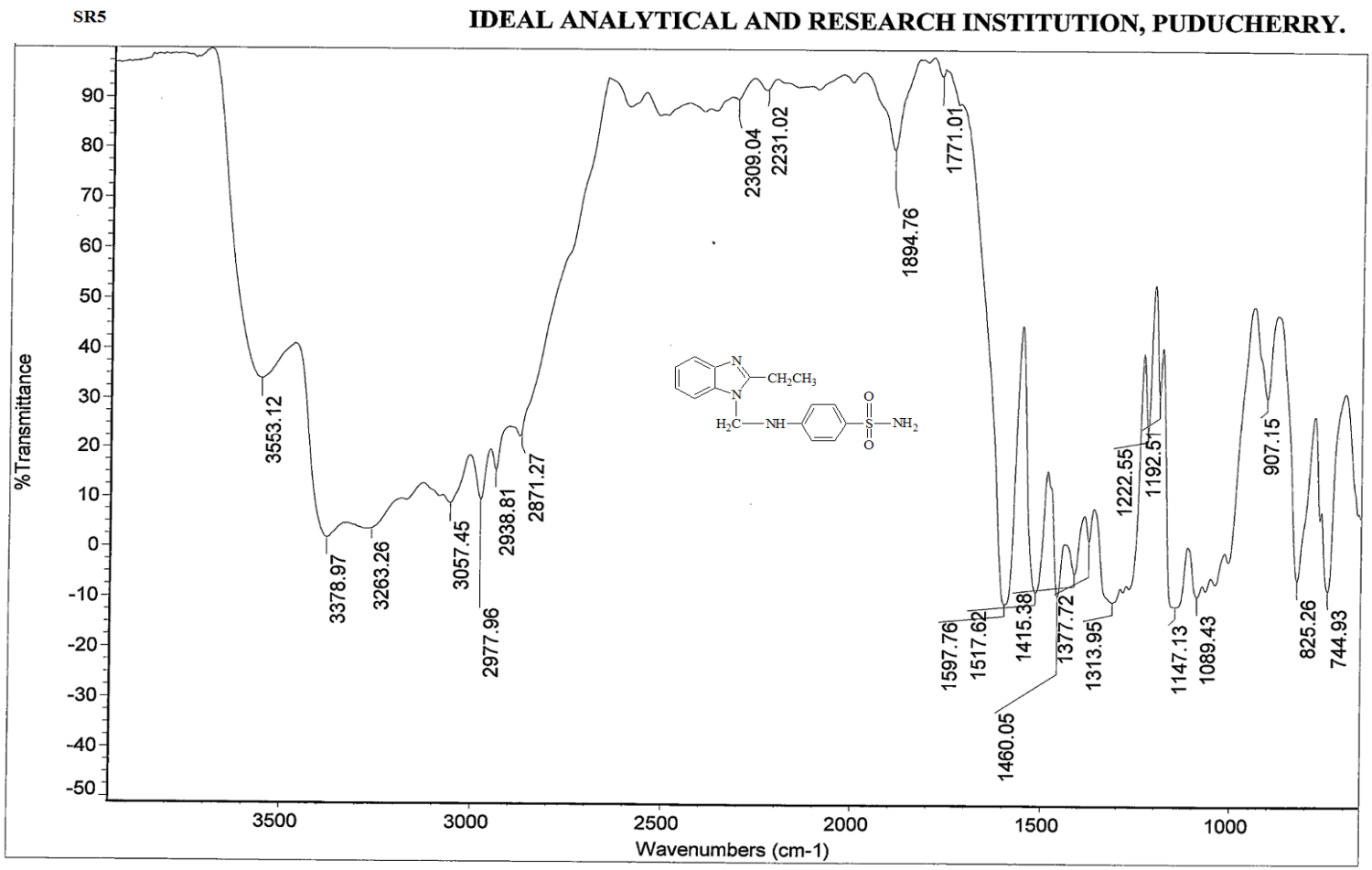


Fig 43: IR spectrum of the compound SR_5

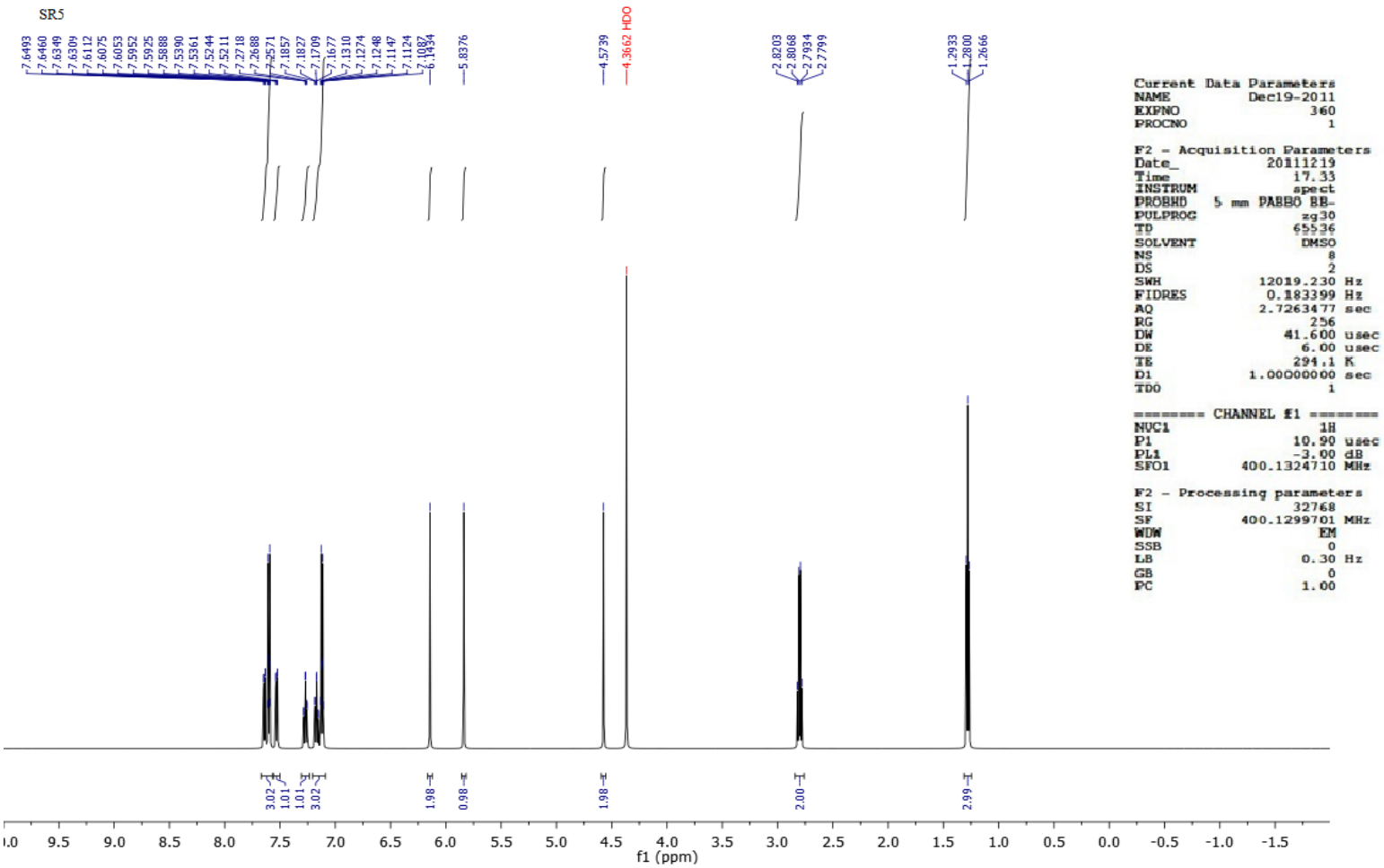


Fig 44: ^{1}H NMR spectrum of the compound SR_5

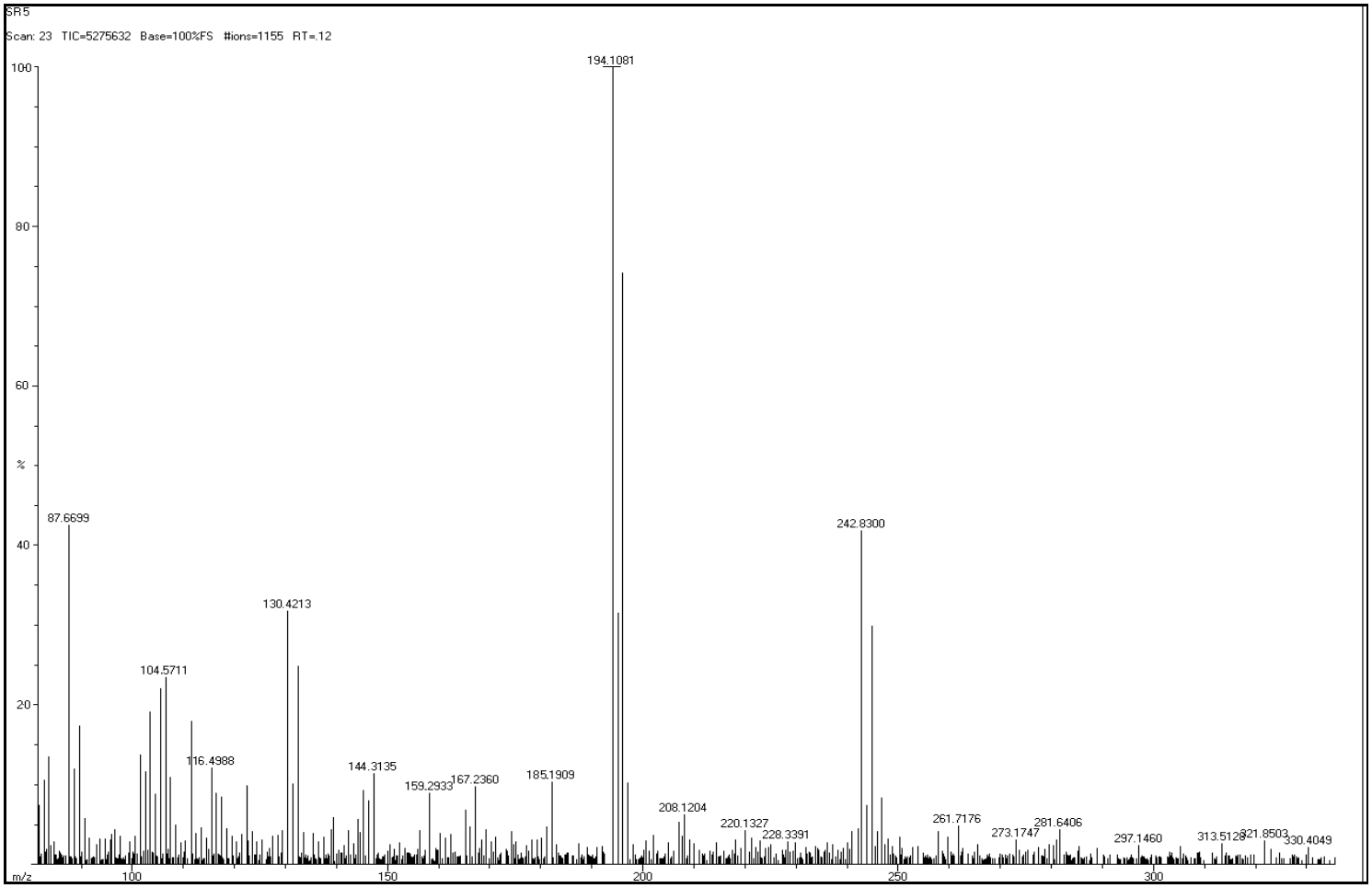


Fig 45: Mass spectrum of the compound SR_5

6.2.6 Spectral analysis of 1-((sulphonomido)-methyl)-2-propyl benzimidazole (SR_6):

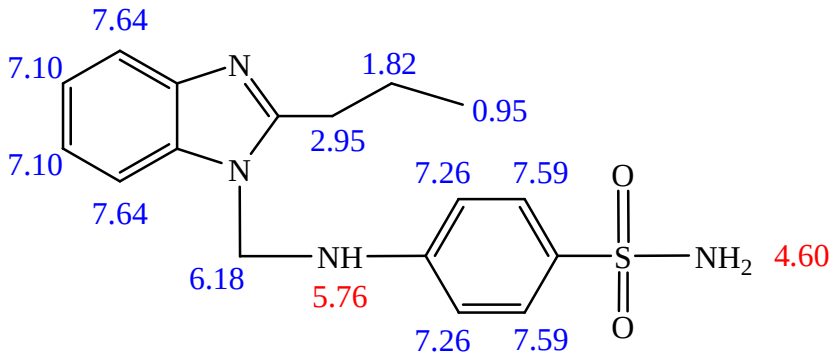
UV (MeOH): (Fig 47)

λ_{max} - 263.0 nm (ε_{max} – 0.7203)

IR (KBr): (Fig 48)

Wave number (cm^{-1})	**Assignment**
3385 (s)	1° N-H stretching in sulphonamide
3289 (s)	2° N-H stretching in sulphonamide
3026 (w)	Aromatic (=C-H) stretching
2931 (m)	C-H stretching in $–CH_2$
1561 (s)	C=N stretching
1458 (s)	C=C stretching
1421 (m)	Aliphatic CH_2 bending
1320 (s)	SO_2 stretching
1383 (s)	C-N stretching (Aromatic tertiary amine)
748 (s)	C-H out-of-planes bending (Aromatic C-H)

NMR (DMSO-*d6*): (Fig 49)

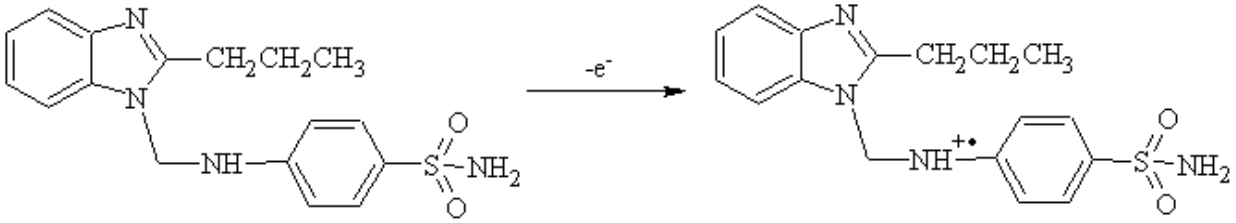


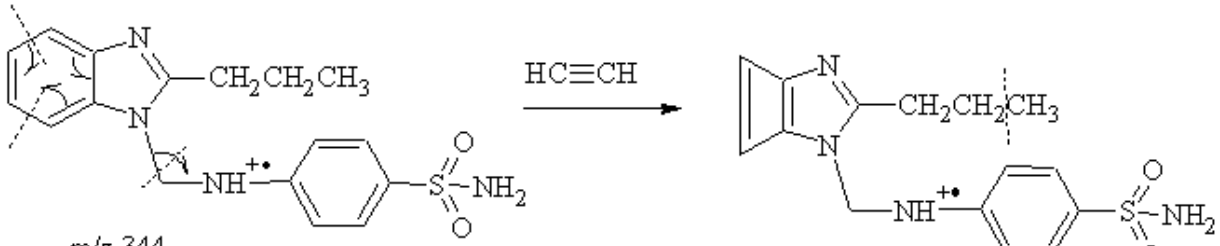
(8 aromatic protons, 9 aliphatic protons and 3 protons on nitrogen)

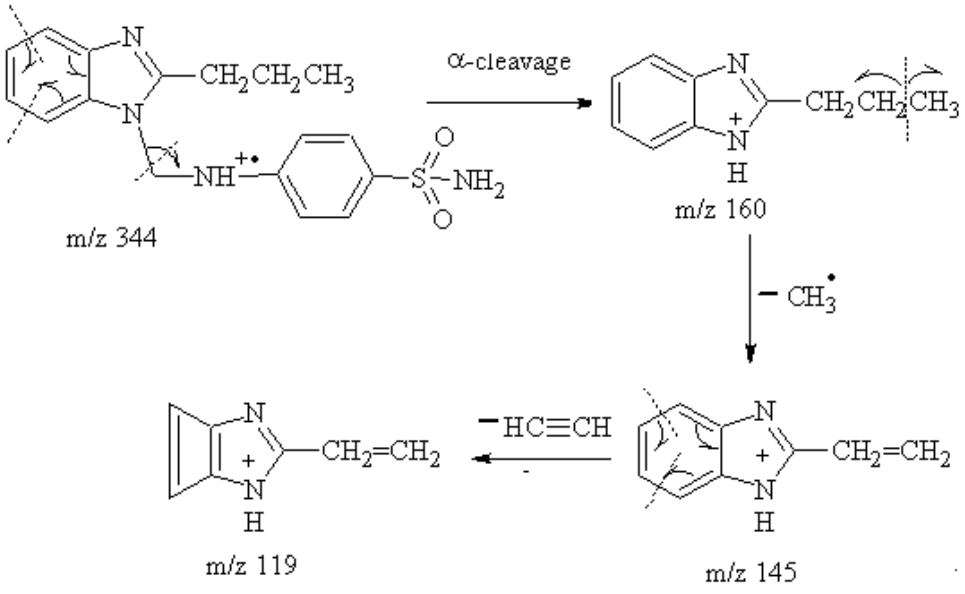
δ – Value	**Assignment**
7.10 – 7.64	(8H, m, Ar-H – C_4, C_5, C_6, C_7 and 4-phenyl protons)
6.18	(2H, s, -CH_2)
5.76	(1H, s, -NH)
4.60	(2H, s, -SO_2NH_2)
2.95	(2H, t, -CH_2)
1.78 – 1.85	(2H, m, -CH_2)
0.95	(3H, t, -CH_3)

Mass: (Fig 50)

The structure of the compound was confirmed by its fragmentation peaks which are as follows:


-e⁻
m/z 344 (M⁺ peak)


HC≡CH
m/z 344
m/z 318


α-cleavage
m/z 344
m/z 160
− CH₃•
− HC≡CH
m/z 119
m/z 145

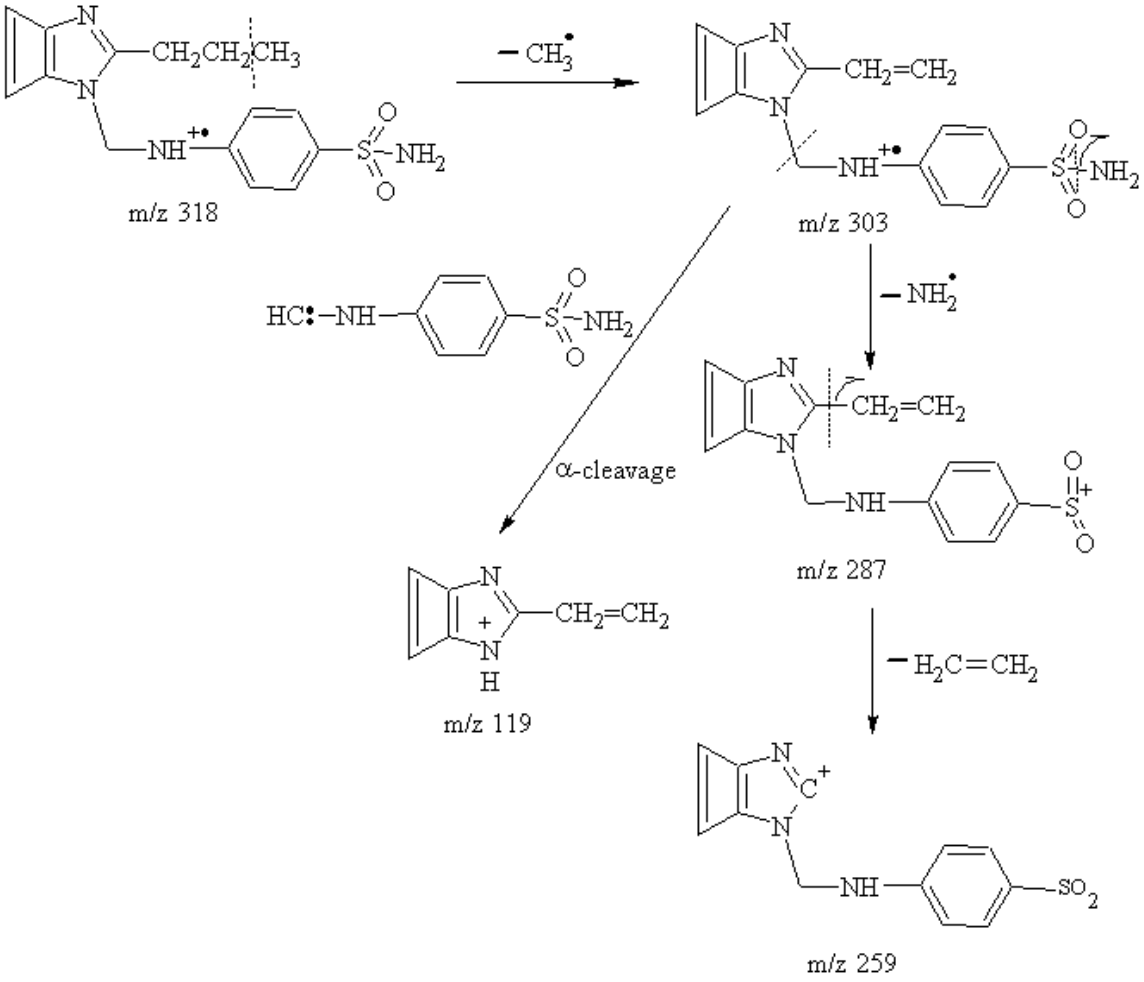
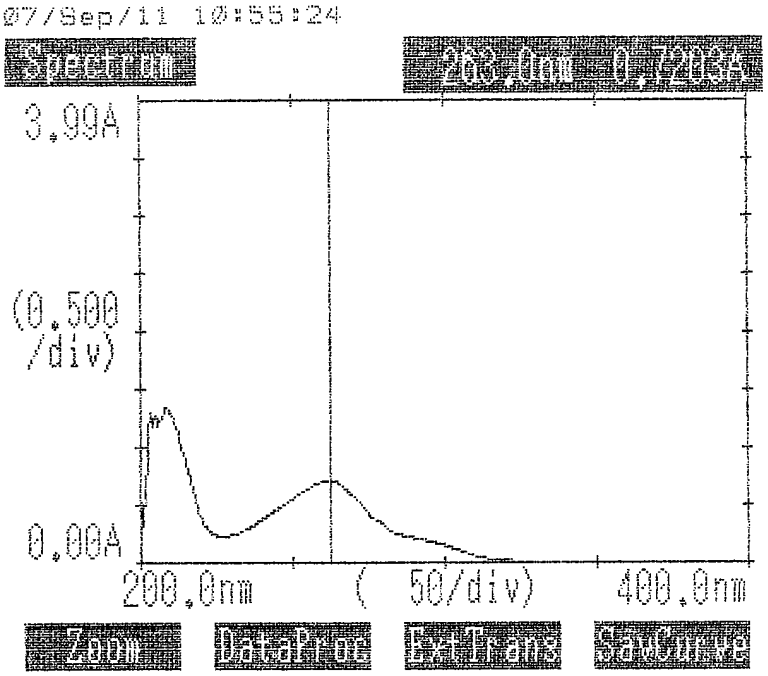


Fig 46: Fragmentation pattern of SR_6



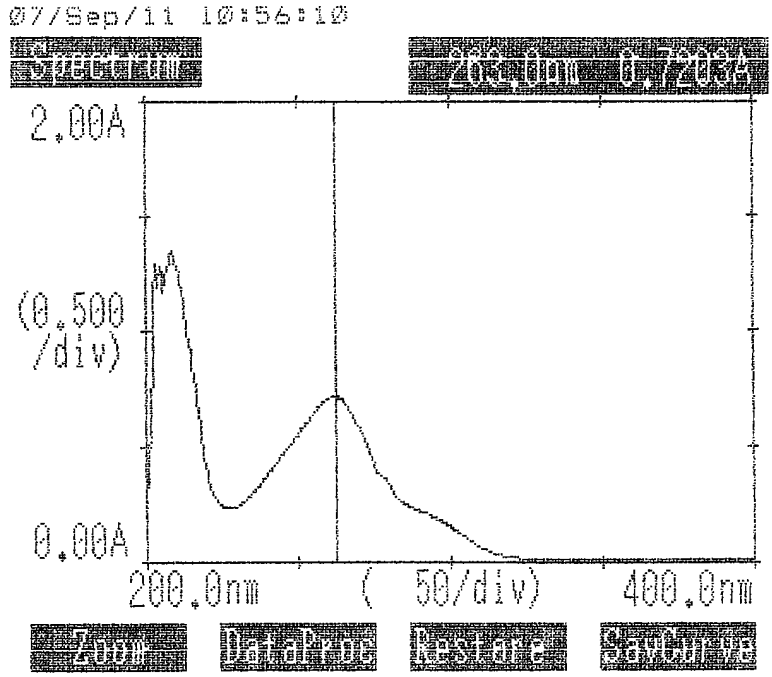


Fig 47: UV spectrum of the compound SR_6

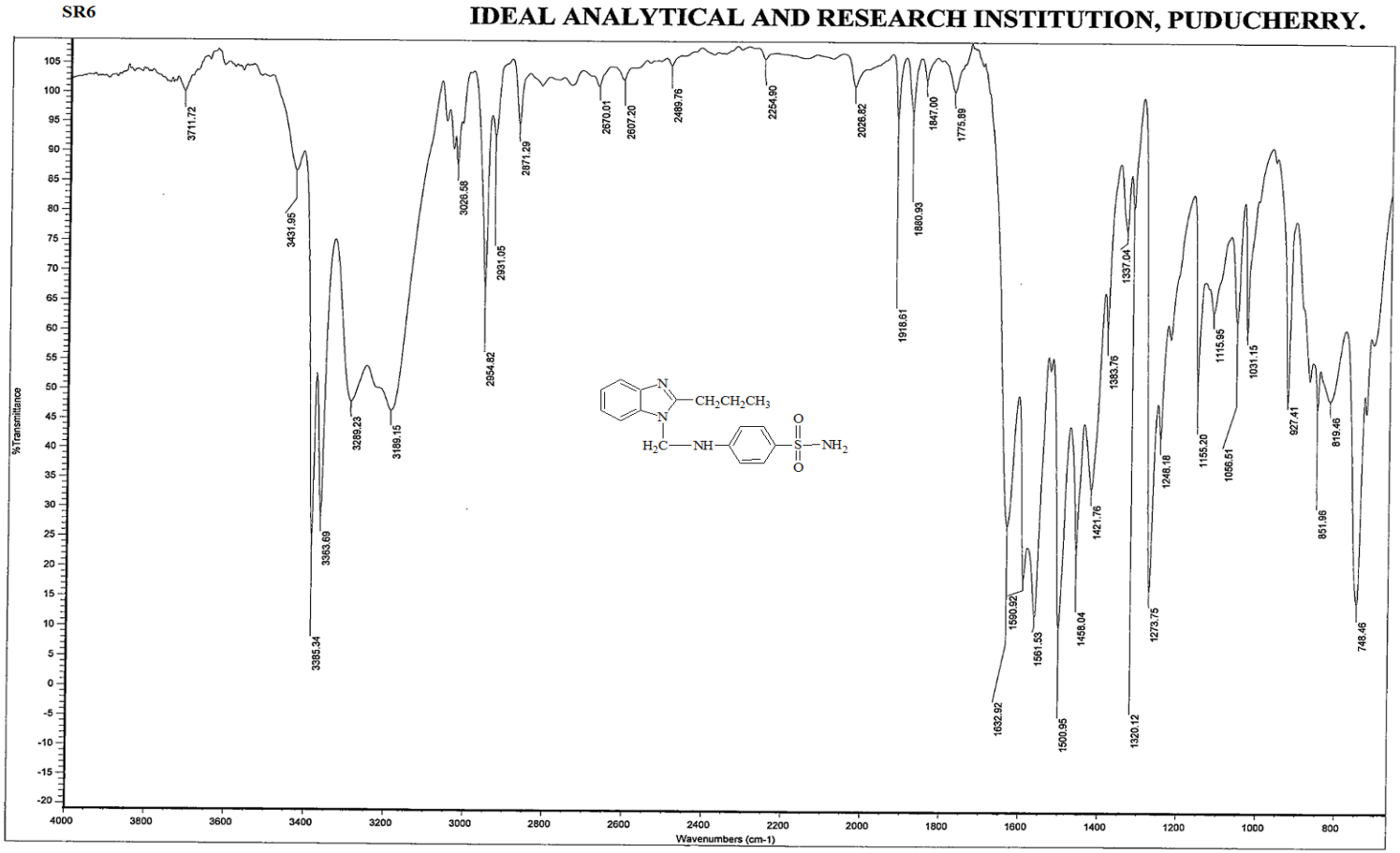


Fig 48: IR spectrum of the compound SR_6

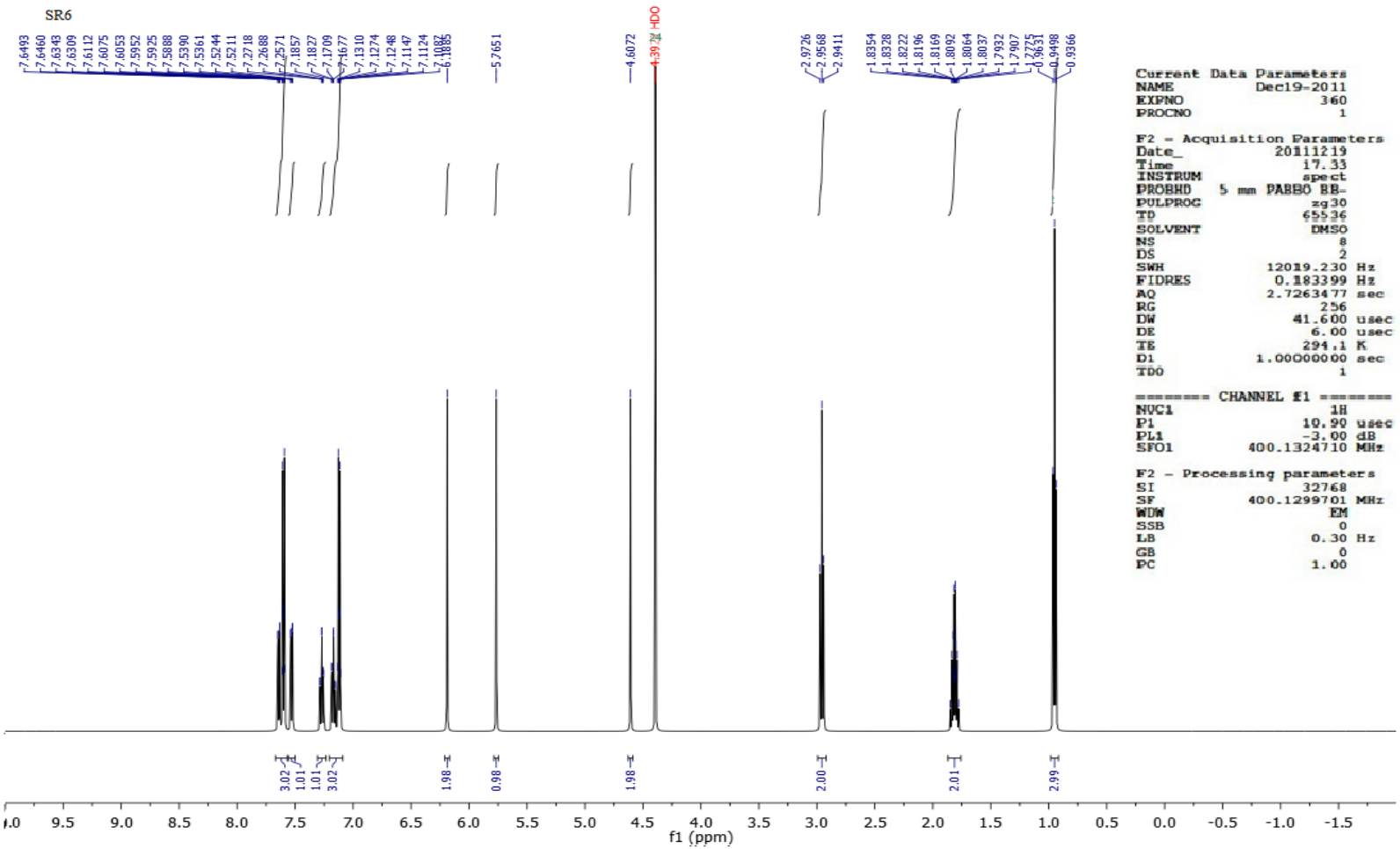


Fig 49: ^{1}H NMR spectrum of the compound SR_6

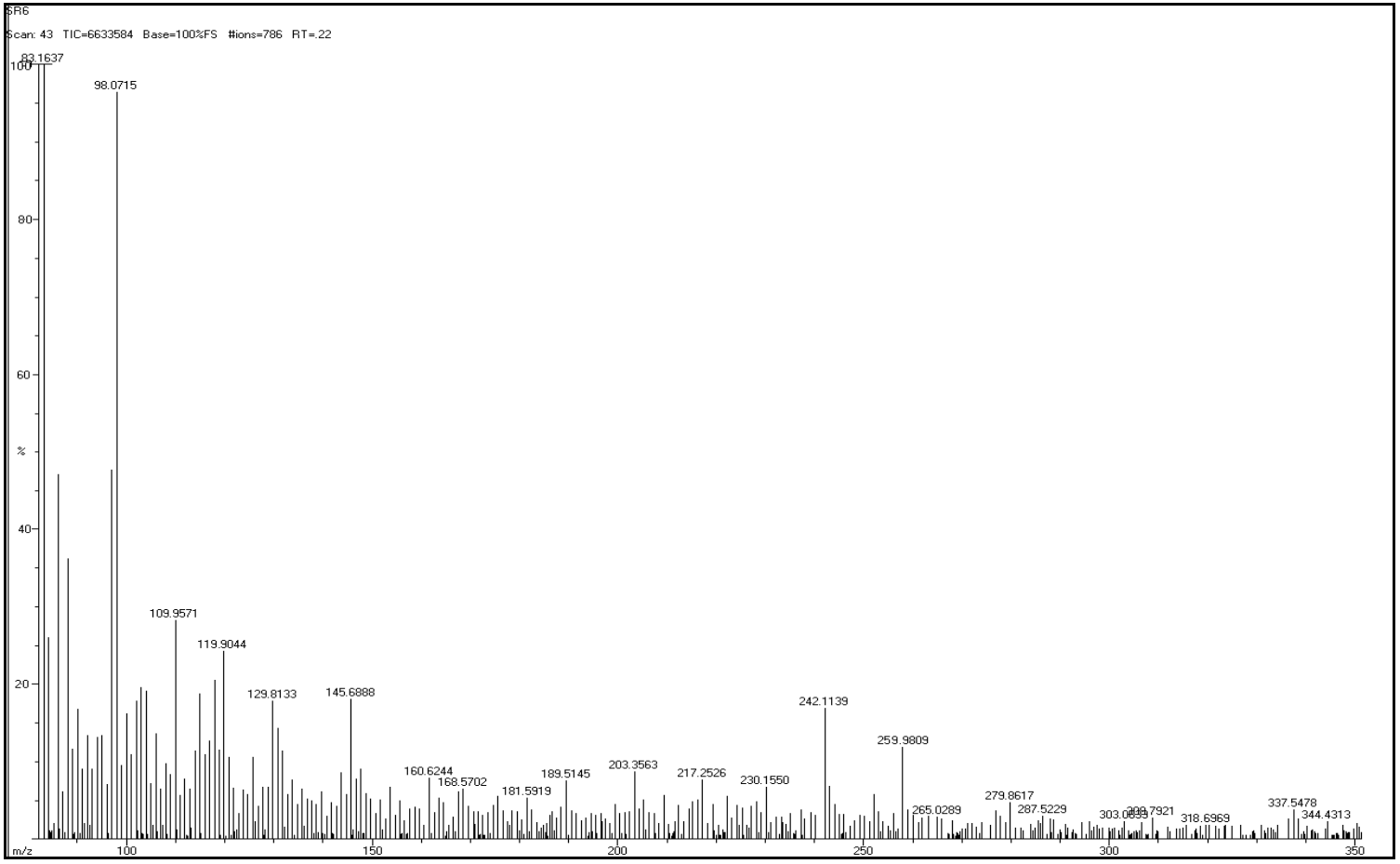


Fig 50: Mass spectrum of the compound SR_6

6.2.7 Spectral analysis of 1-((piperazino)-methyl)-2-methyl benzimidazole (SR_7):

UV (MeOH): (Fig 52)

λ_{max} - 280.0 nm (ε_{max} – 0.3619)

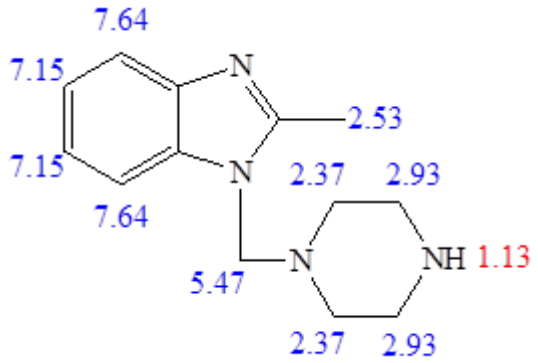
λ_{max} - 273.5 nm (ε_{max} – 0.2915)

λ_{max} - 237.5 nm (ε_{max} – 0.3762)

IR (KBr): (Fig 53)

Wave number (cm^{-1})	**Assignment**
3409 (w)	2° N-H stretching in piperazine
3063 (w)	Aromatic (=C-H) stretching
2933 (m)	C-H stretching in CH_2 group
2874 (m)	C-H stretching in piperazine
1622 (s)	C=N stretching
1450 (s)	C=C stretching
1415 (s)	CH_2 asymmetric bending
1346 (s)	C-N stretching (Aromatic tertiary amine)
736 (s)	C-H out-of-plane bending (Aromatic C-H)

NMR (DMSO-*d6*): (Fig 54)

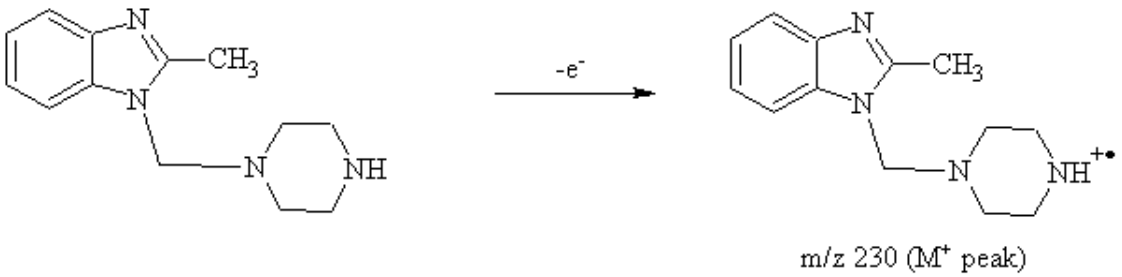


(4 aromatic protons, 13 aliphatic protons and 1 proton on nitrogen)

δ – Value	**Assignment**
7.15 – 7.64	(4H, m, Ar-H – C_4, C_5, C_6 and C_7)
5.47	(2H, s, -CH_2)
2.53	(3H, s, -CH_3)
2.37 – 2.93	(8H, m, CH_2 of piperazine)
1.13	(1H, s, -NH)

Mass: (Fig 55)

The structure of the compound was confirmed by its fragmentation peaks which are as follows:



m/z 230 (M^+ peak)

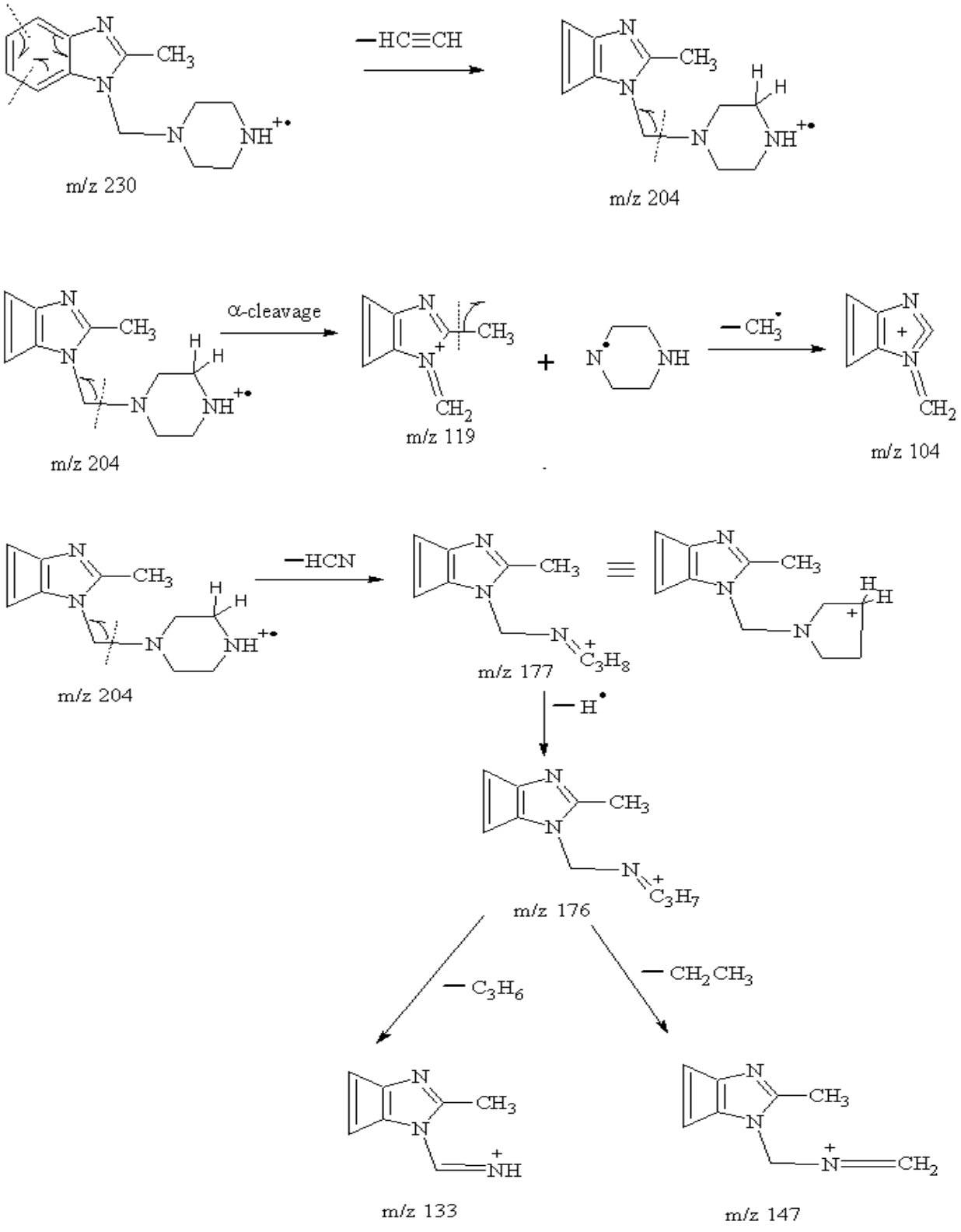


Fig 51: Fragmentation pattern of SR_7

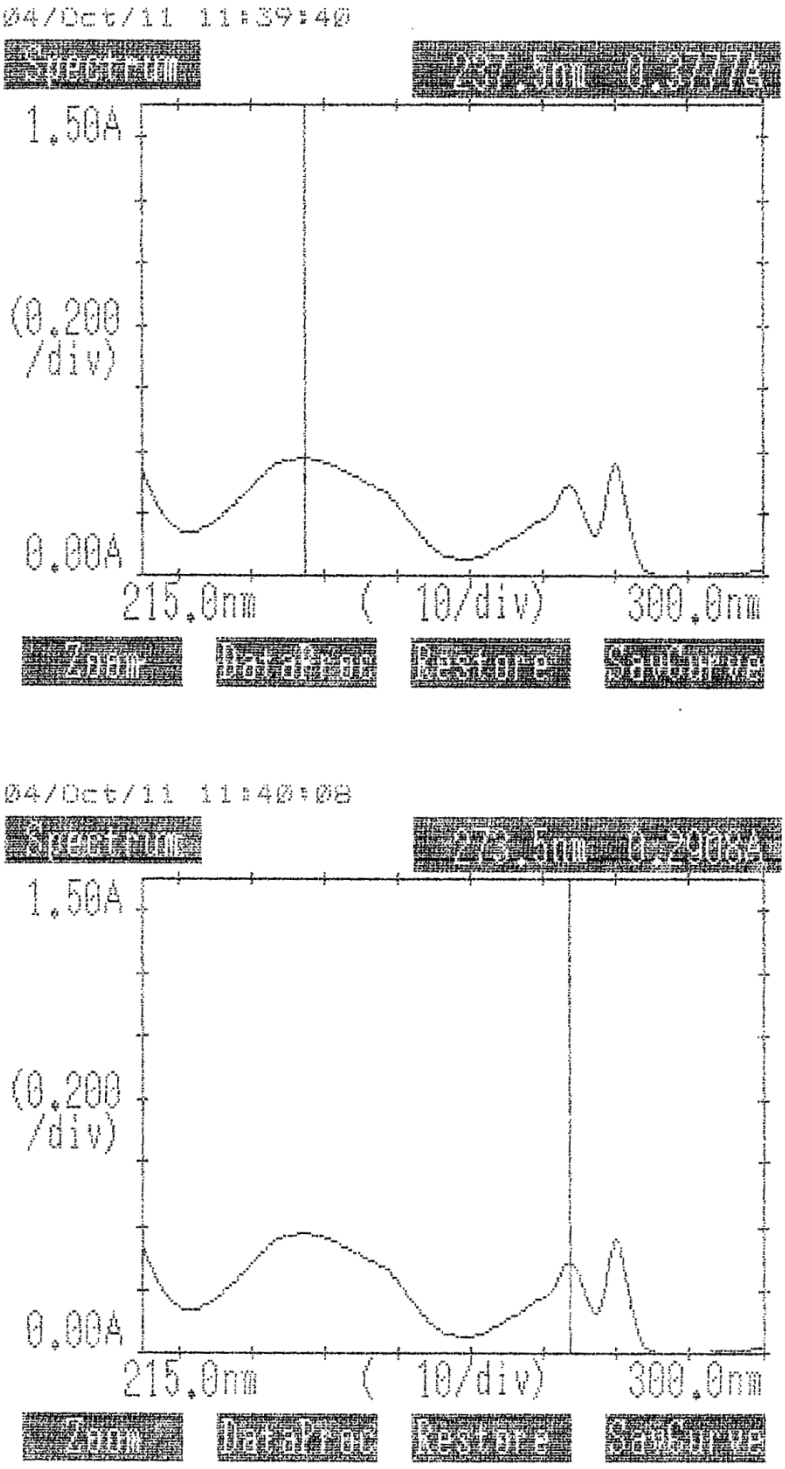


Fig 52: UV spectrum of the compound SR_7

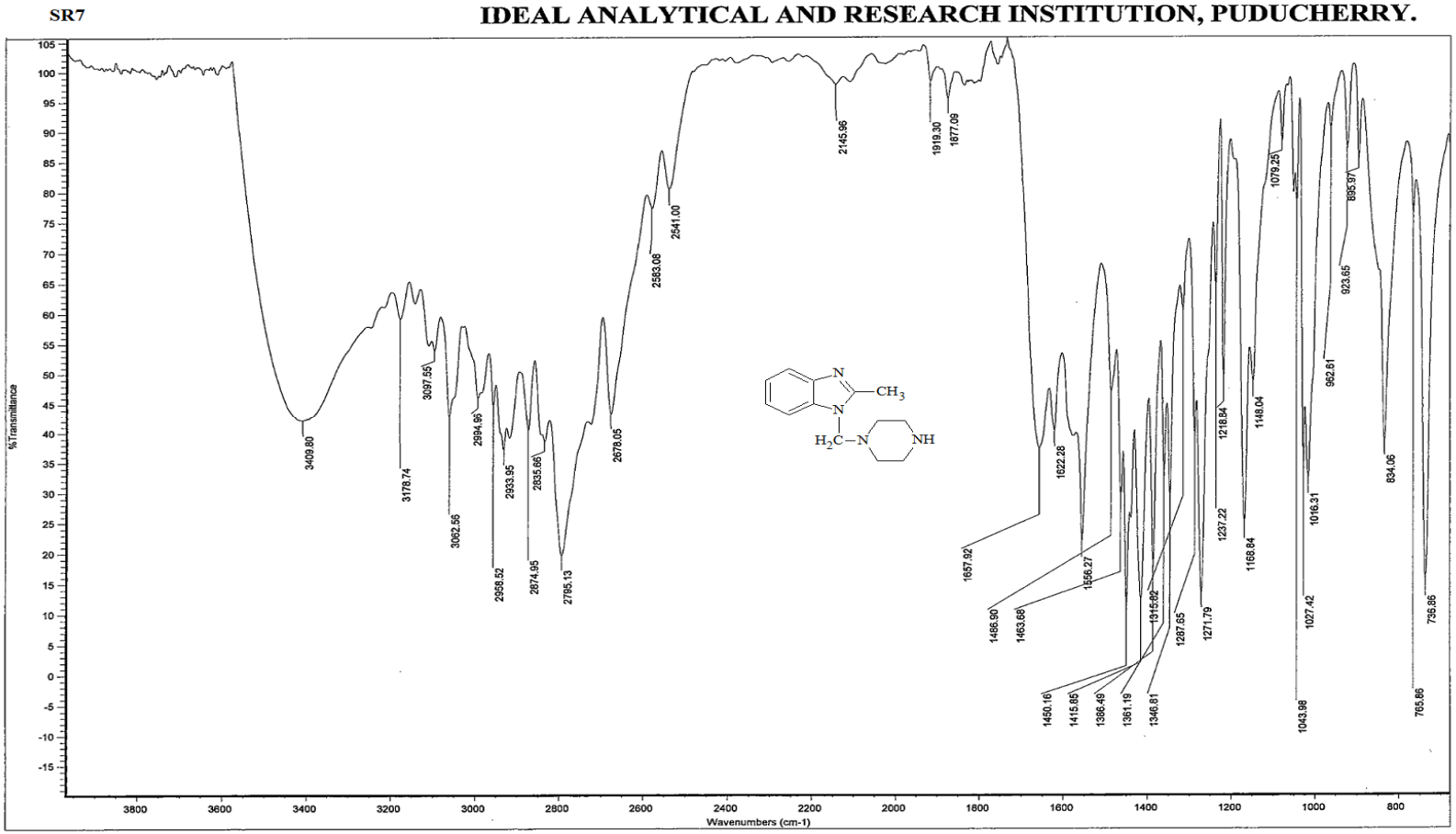


Fig 53: IR spectrum of the compound SR_7

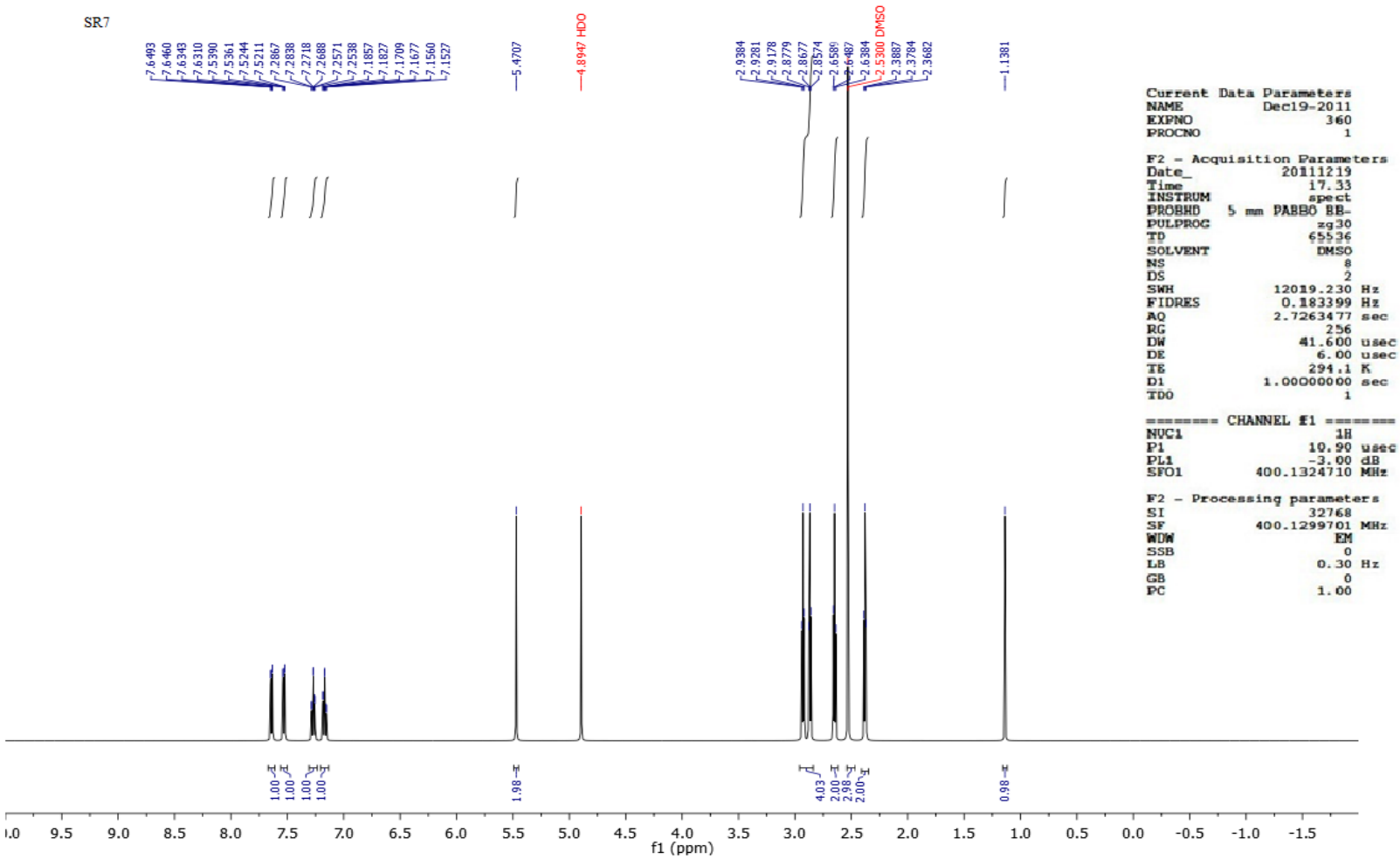


Fig 54: ^{1}H NMR spectrum of the compound SR_7

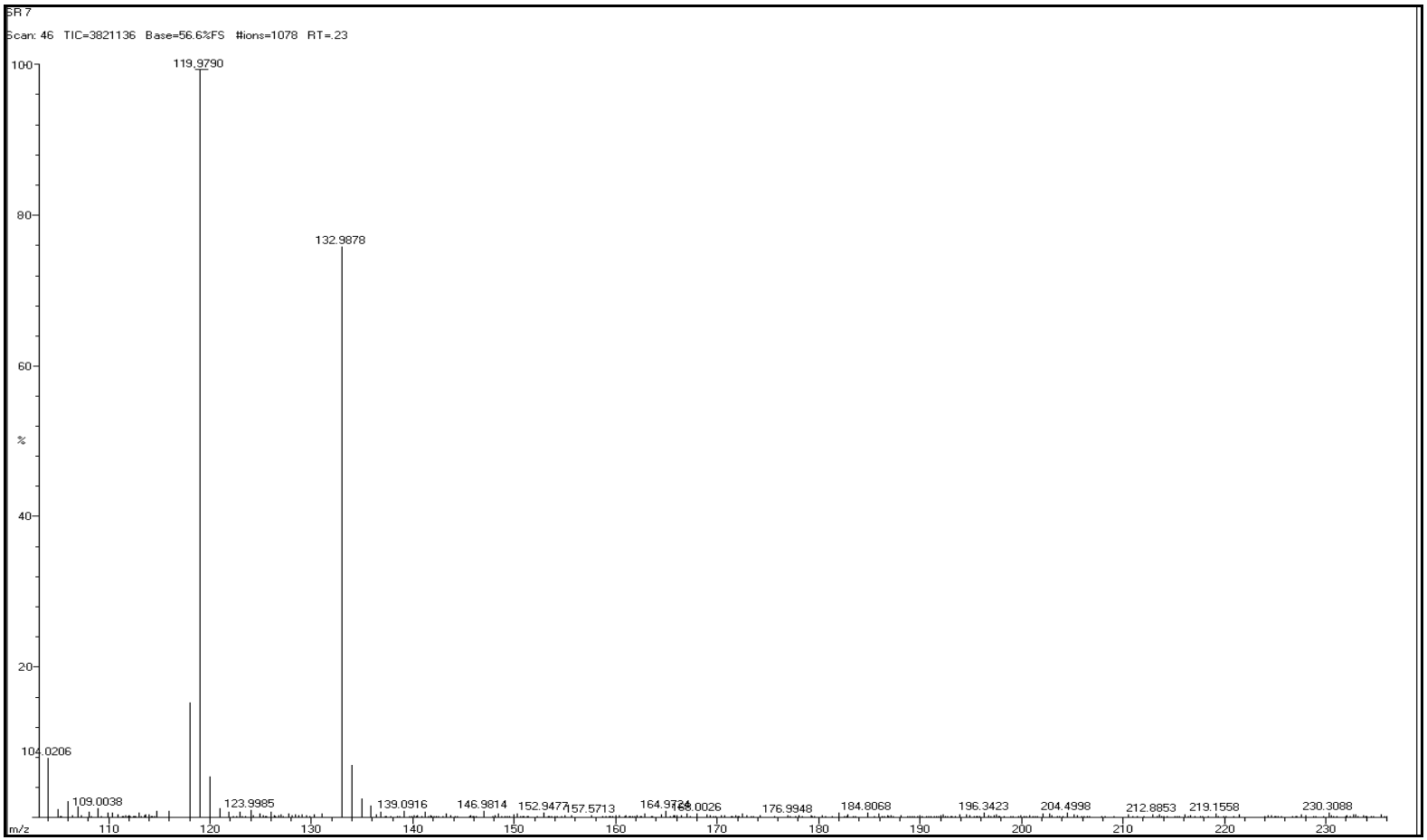


Fig 55: Mass spectrum of the compound SR_7

6.2.8 Spectral analysis of 1-((piperazino)-methyl)-2-ethyl benzimidazole (SR_8):

UV (MeOH): (Fig 57)

λ_{max} - 280.5 nm (ε_{max} – 0.2572)

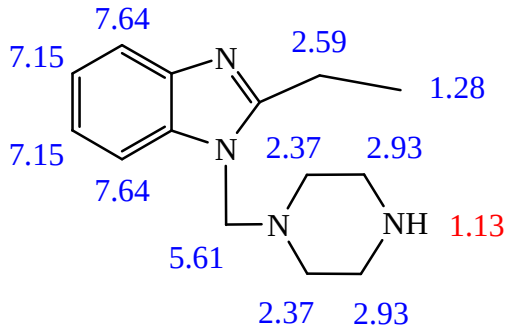
λ_{max} - 274.5 nm (ε_{max} – 0.2415)

λ_{max} - 242.5 nm (ε_{max} – 0.2018)

IR (KBr): (Fig 58)

Wave number (cm^{-1})	**Assignment**
3416 (w)	2° N-H stretching in piperazine
3053 (w)	Aromatic (=C-H) stretching
2936 (m)	C-H stretching in CH_2 group
2796 (m)	C-H stretching in piperazine
1622 (s)	C=N stretching
1429 (s)	CH_2 asymmetric bending
1347 (s)	C-N stretching (Aromatic tertiary amine)
741 (s)	C-H out-of-plane bending (Aromatic C-H)

NMR (DMSO-*d6*): (Fig 59)

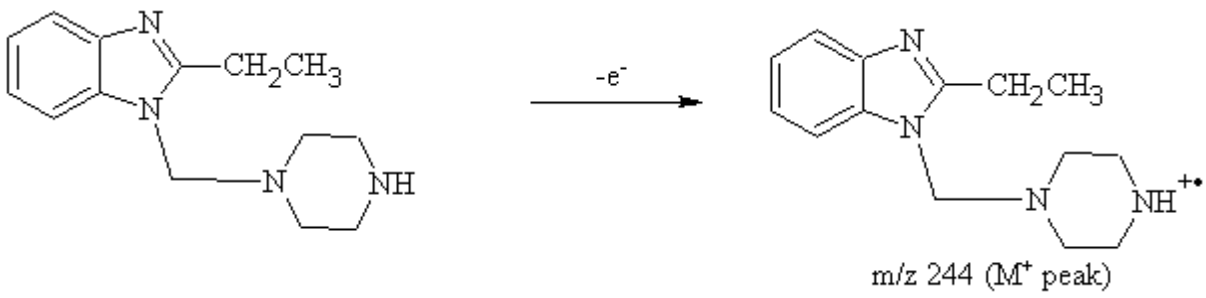


(4 aromatic protons, 15 aliphatic protons and 1 proton on nitrogen)

δ – Value	**Assignment**
7.15 – 7.64	(4H, m, Ar-H – C_4, C_5, C_6, C_7)
5.61	(2H, s, -CH_2)
2.37 - 2.93	(10H, m, CH_2 of piperazine and CH_2 of ethyl group)
1.28	(3H, t, -CH_3)
1.13	(1H, s, -NH)

Mass: (Fig 60)

The structure of the compound was confirmed by its fragmentation peaks which are as follows:



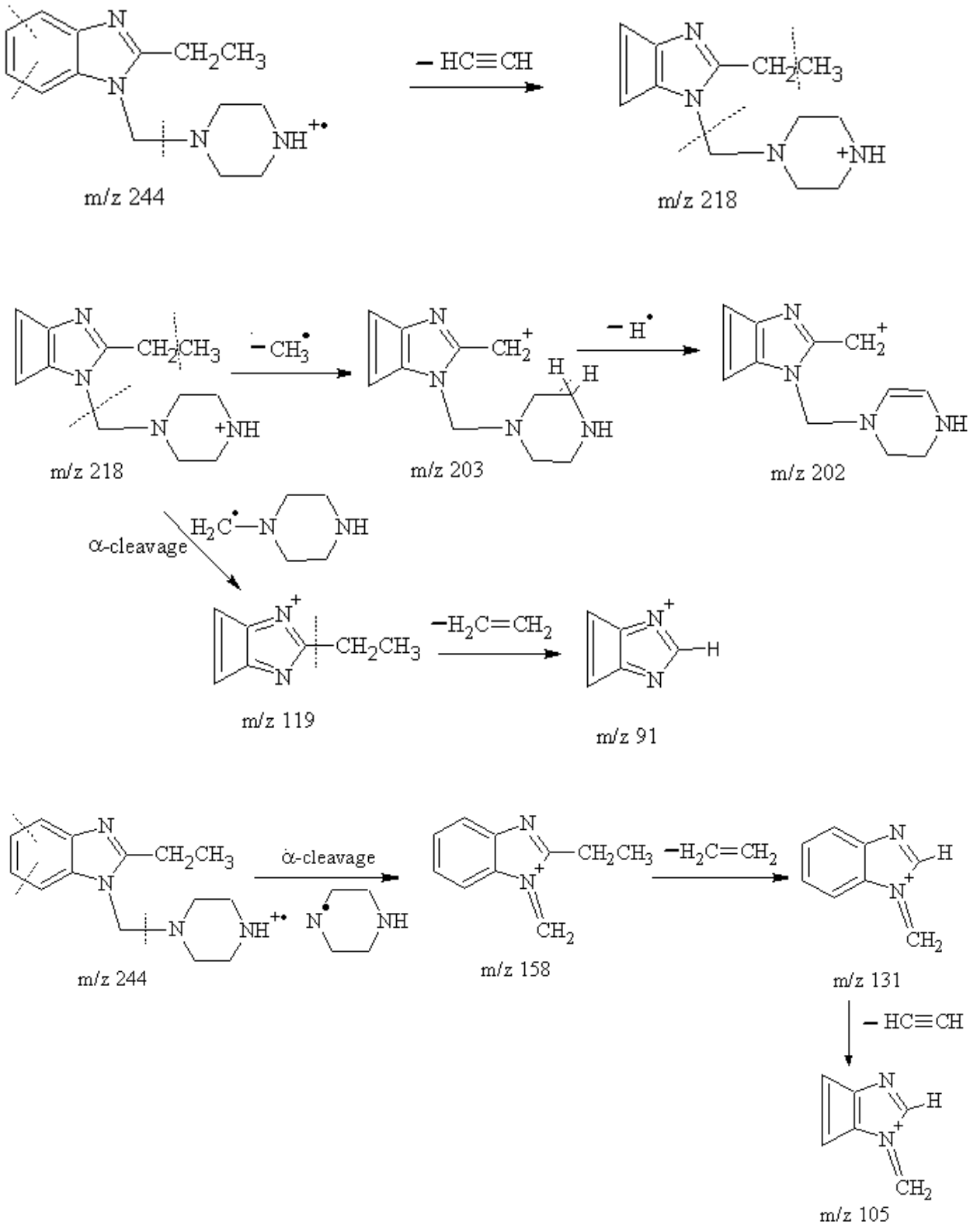


Fig 56: Fragmentation pattern of SR_8

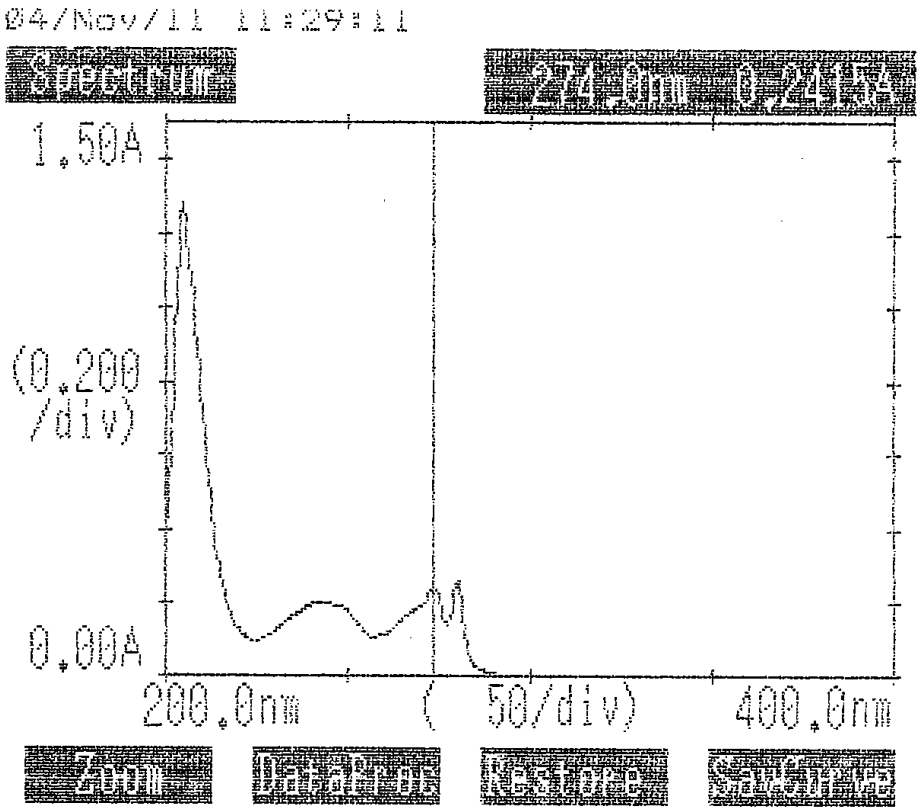


Fig 57: UV spectrum of the compound SR_8

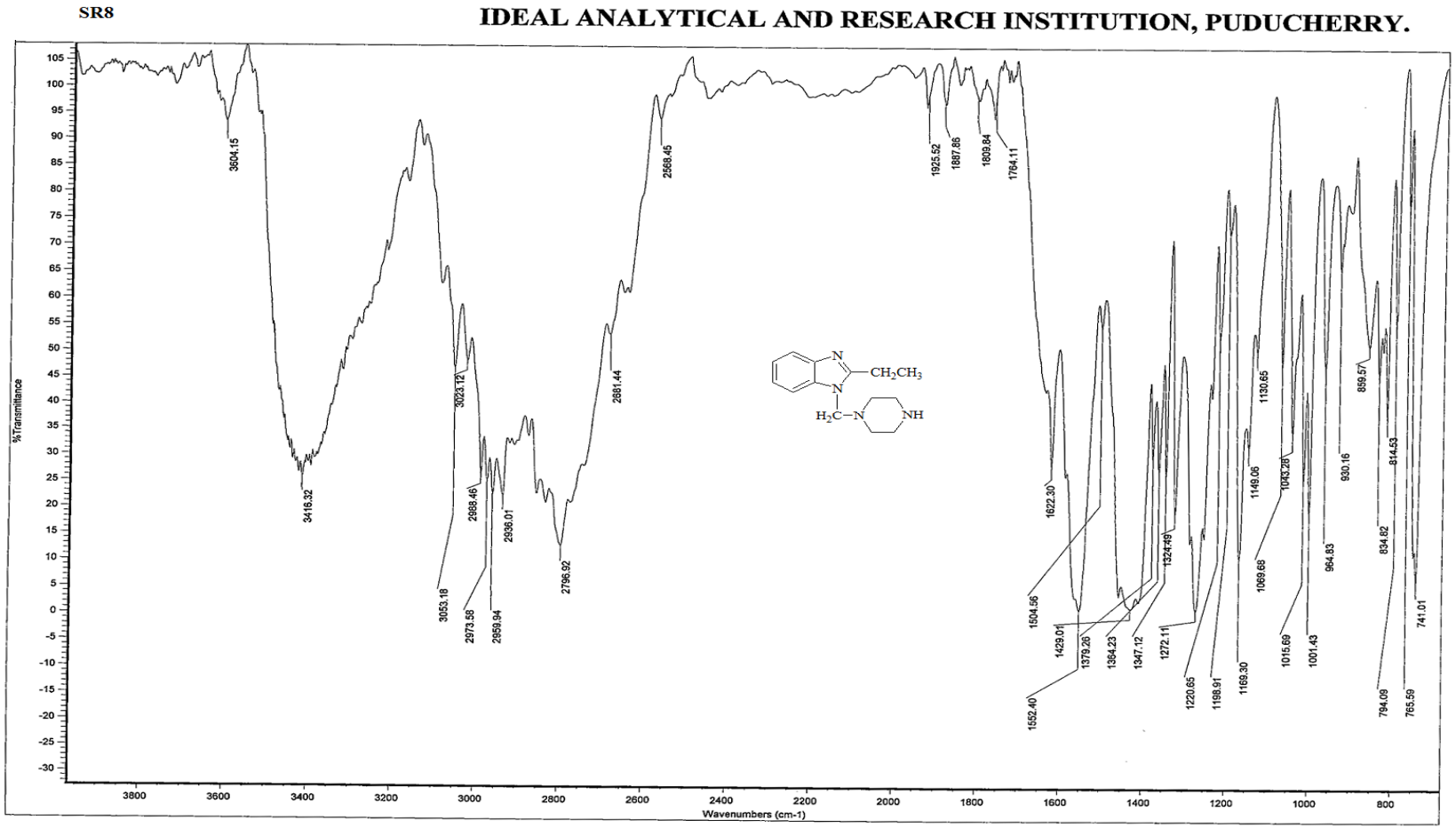


Fig 58: IR spectrum of the compound SR_8

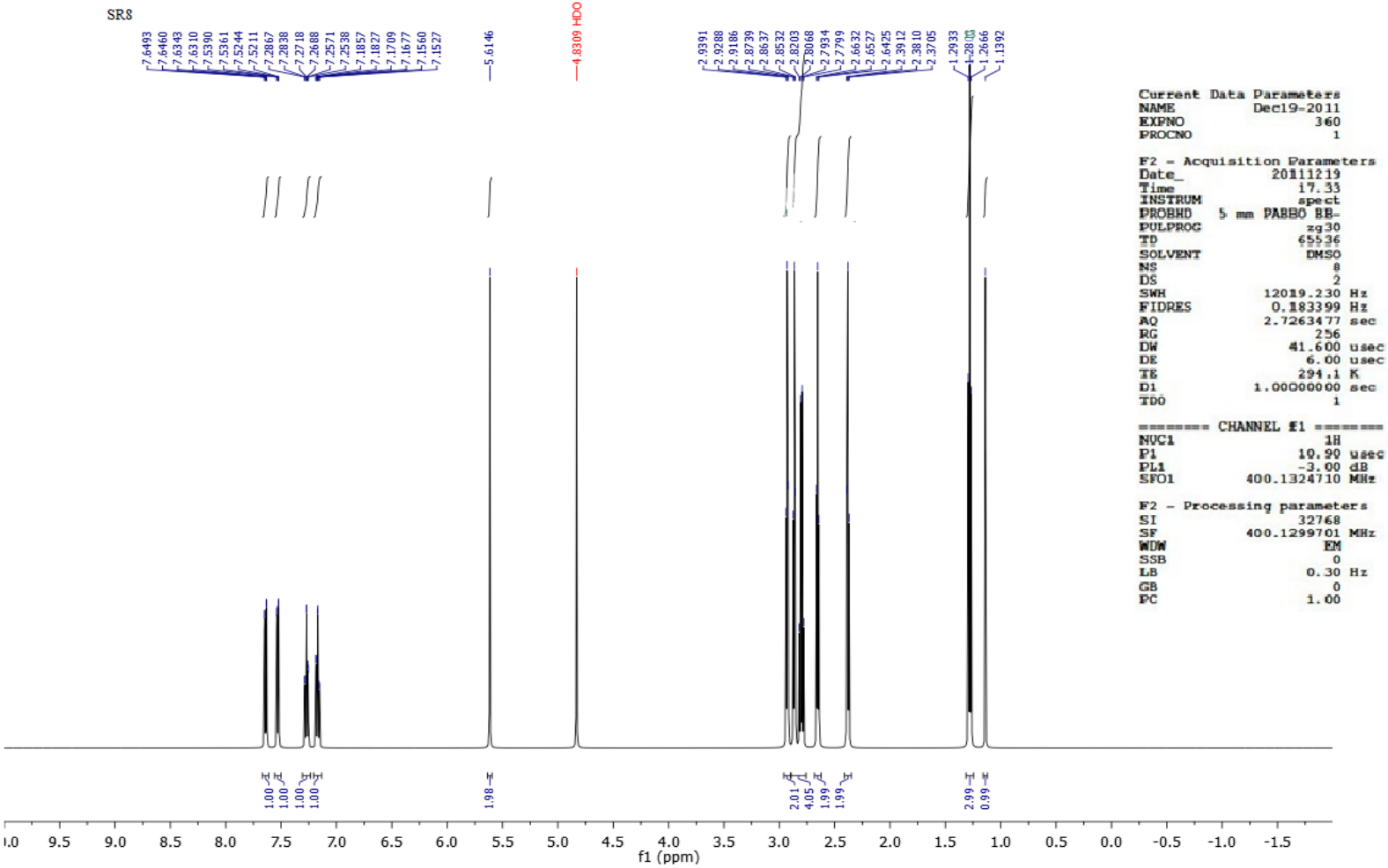


Fig 59: ^{1}H NMR spectrum of the compound SR_8

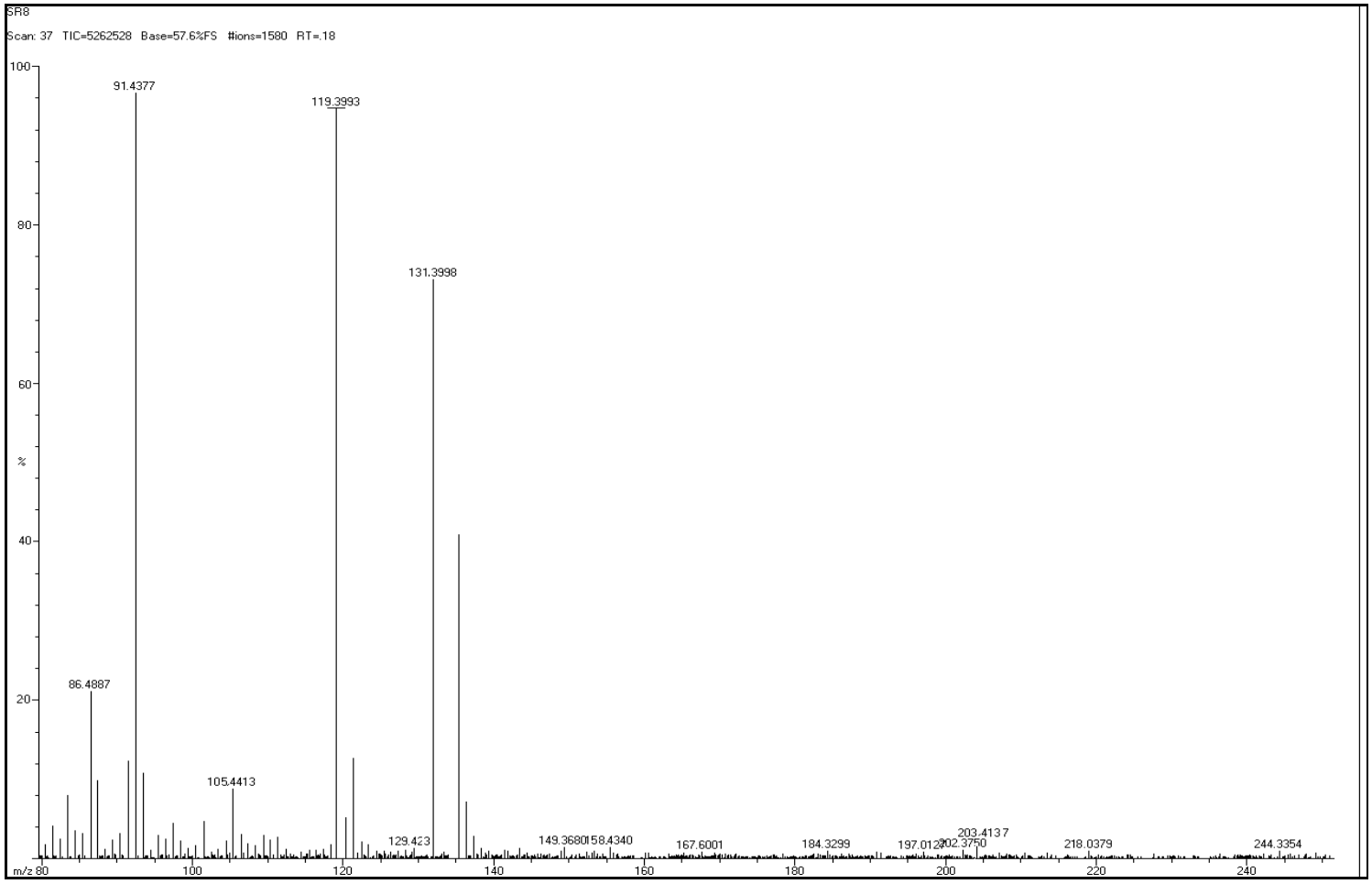


Fig 60: Mass spectrum of the compound SR_8

6.2.9 Spectral analysis of 1-((piperazino)-methyl)-2-propyl benzimidazole (SR_9):

UV (MeOH): (Fig 62)

λ_{max} - 280.5 nm (ε_{max} – 0.2052)

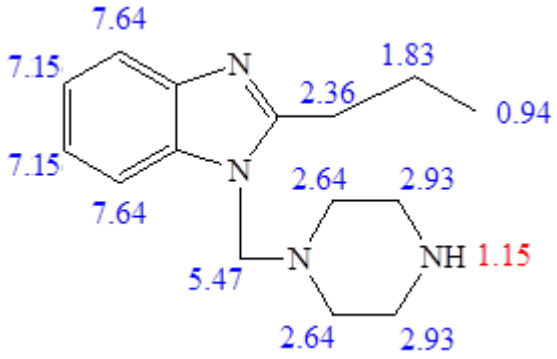
λ_{max} - 274.0 nm (ε_{max} – 0.1995)

λ_{max} - 233.5 nm (ε_{max} – 0.4237)

IR (KBr): (Fig 63)

Wave number (cm^{-1})	**Assignment**
3385 (w)	2° N-H stretching in piperazine
3026 (w)	Aromatic (=C-H) stretching
2934 (m)	C-H stretching in CH_2 group
2874 (m)	C-H stretching in piperazine
1631 (s)	C=N stretching
1430 (m)	CH_2 asymmetric bending
1347 (s)	C-N stretching (Aromatic tertiary amine)
749 (s)	C-H out-of-plane bending (Aromatic C-H)

NMR (DMSO-*d6*): (Fig 64)



(4 aromatic protons, 17 aliphatic protons and 1 proton on nitrogen)

δ – Value	**Assignment**
7.15 – 7.64	(4H, m, Ar-H – C_4, C_5, C_6 and C_7)
5.47	(2H, s, -CH_2)
2.64 – 2.93	(8H, m, CH_2 of piperazine)
2.36	(2H, t, -CH_2)
1.81 – 1.84	(2H, m, -CH_2)
1.15	(1H, s, -NH)
0.94	(3H, t, -CH_3)

Mass: (Fig 65)

The structure of the compound was confirmed by its fragmentation peaks which are as follows:

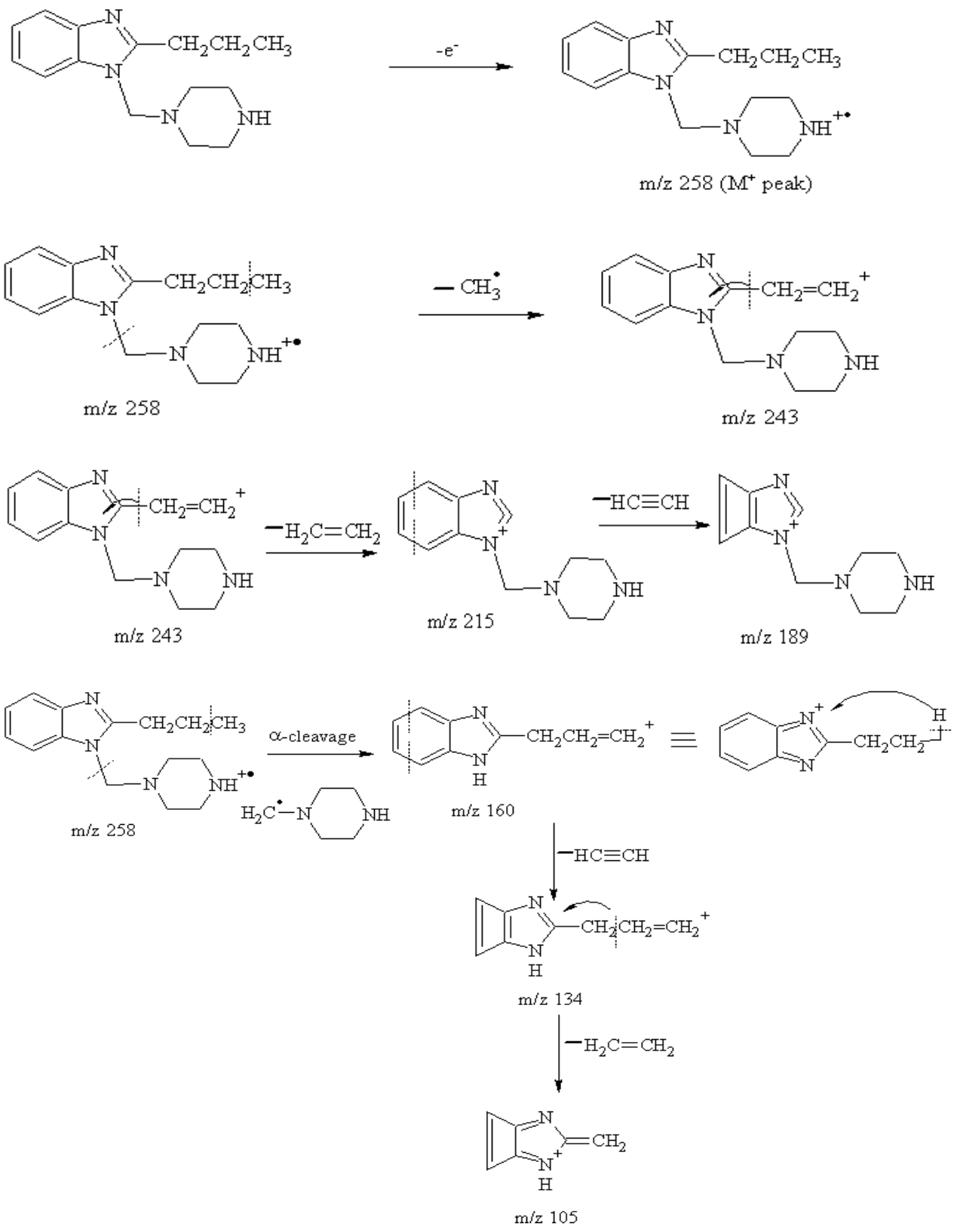
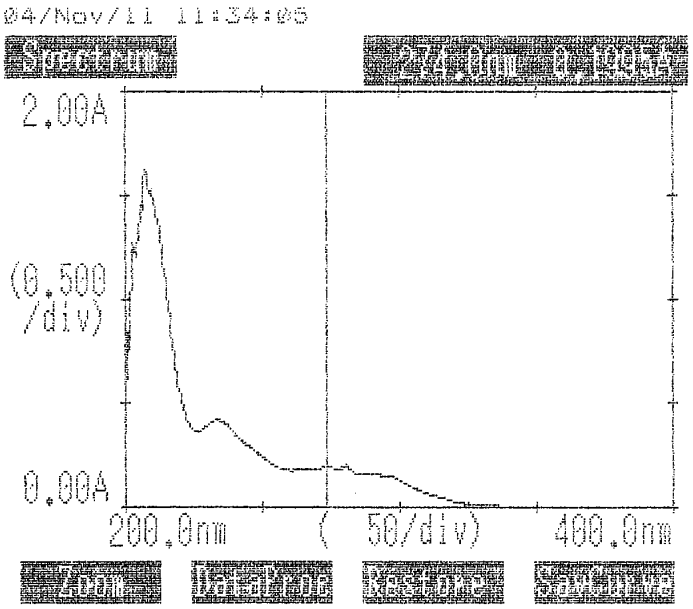


Fig 61: Fragmentation pattern of SR_9



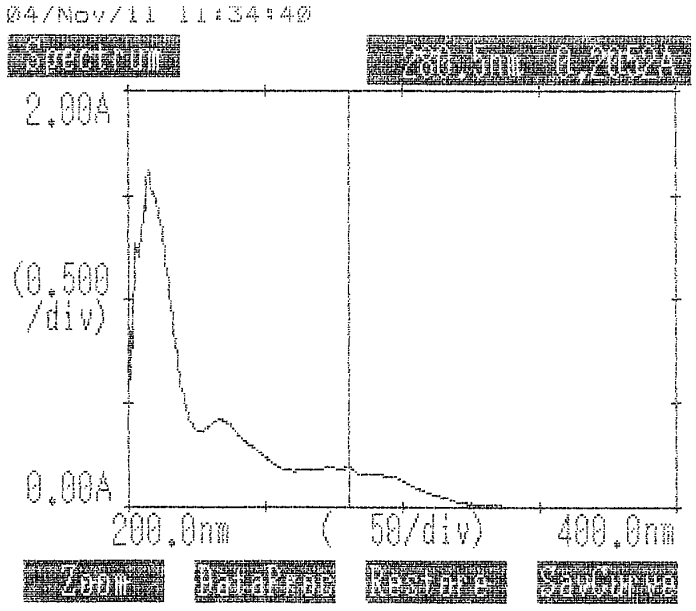


Fig 62: UV spectrum of the compound SR_9

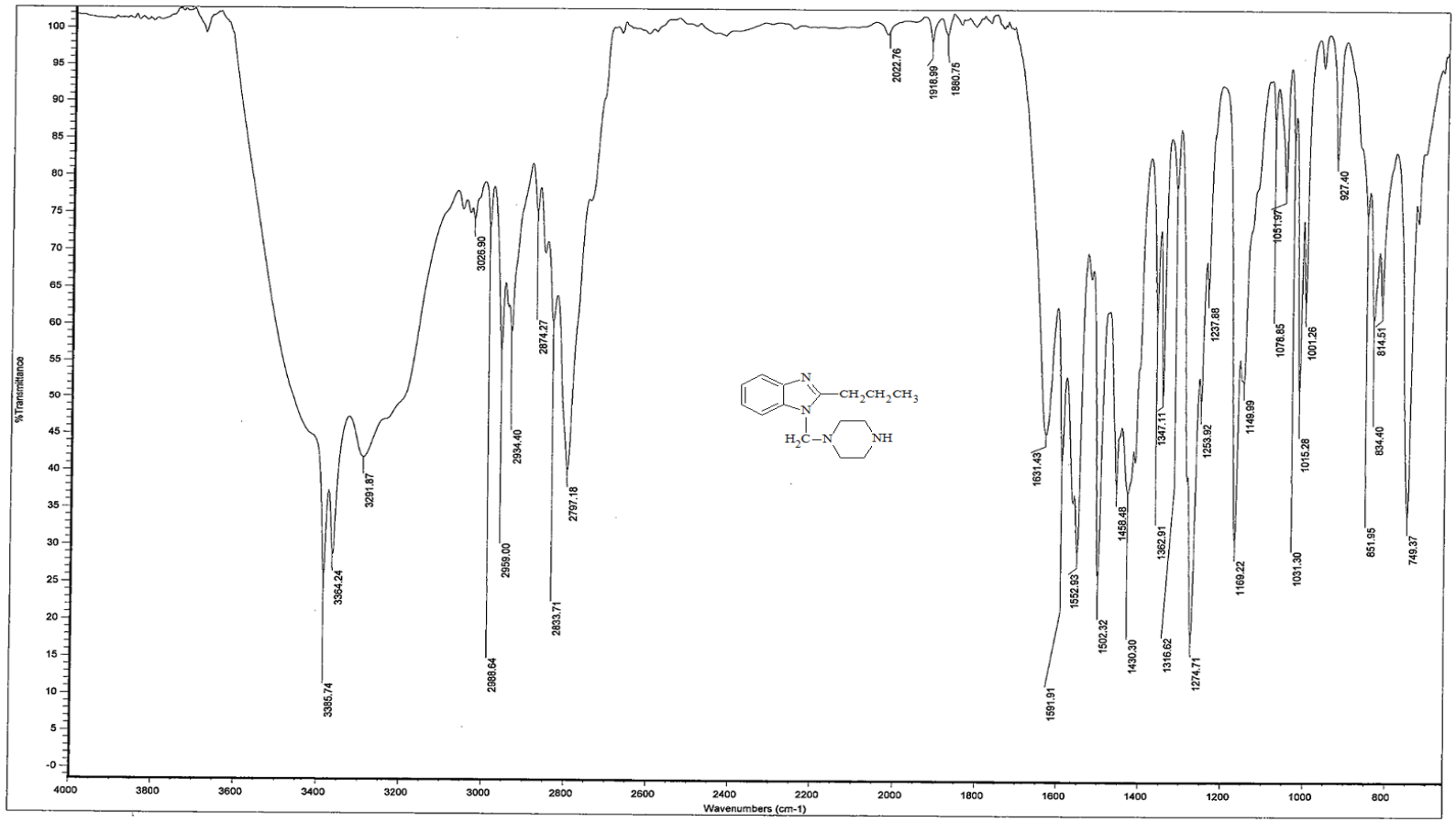


Fig 63: IR spectrum of the compound SR_9

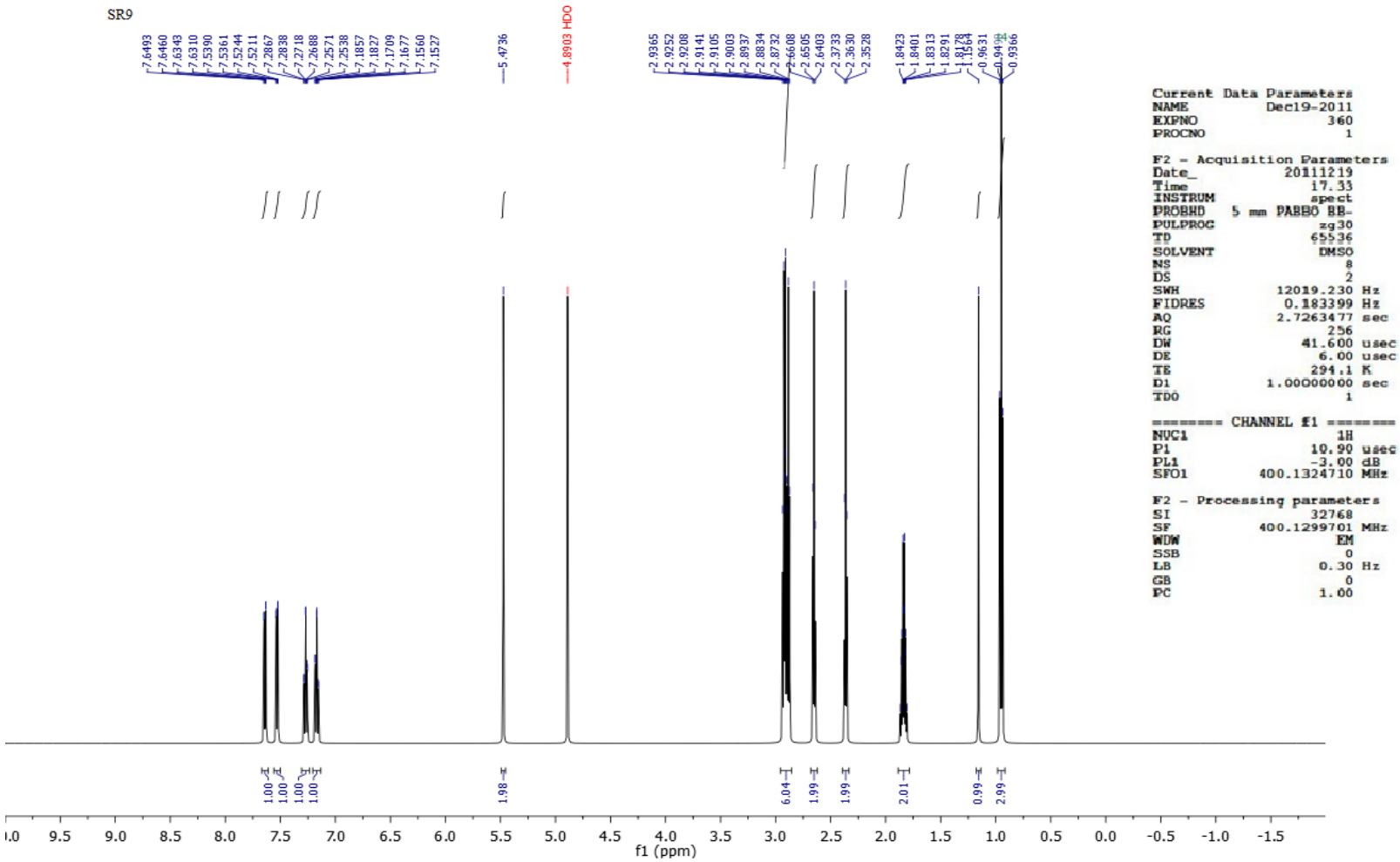


Fig 64: ^{1}H NMR spectrum of the compound SR_9

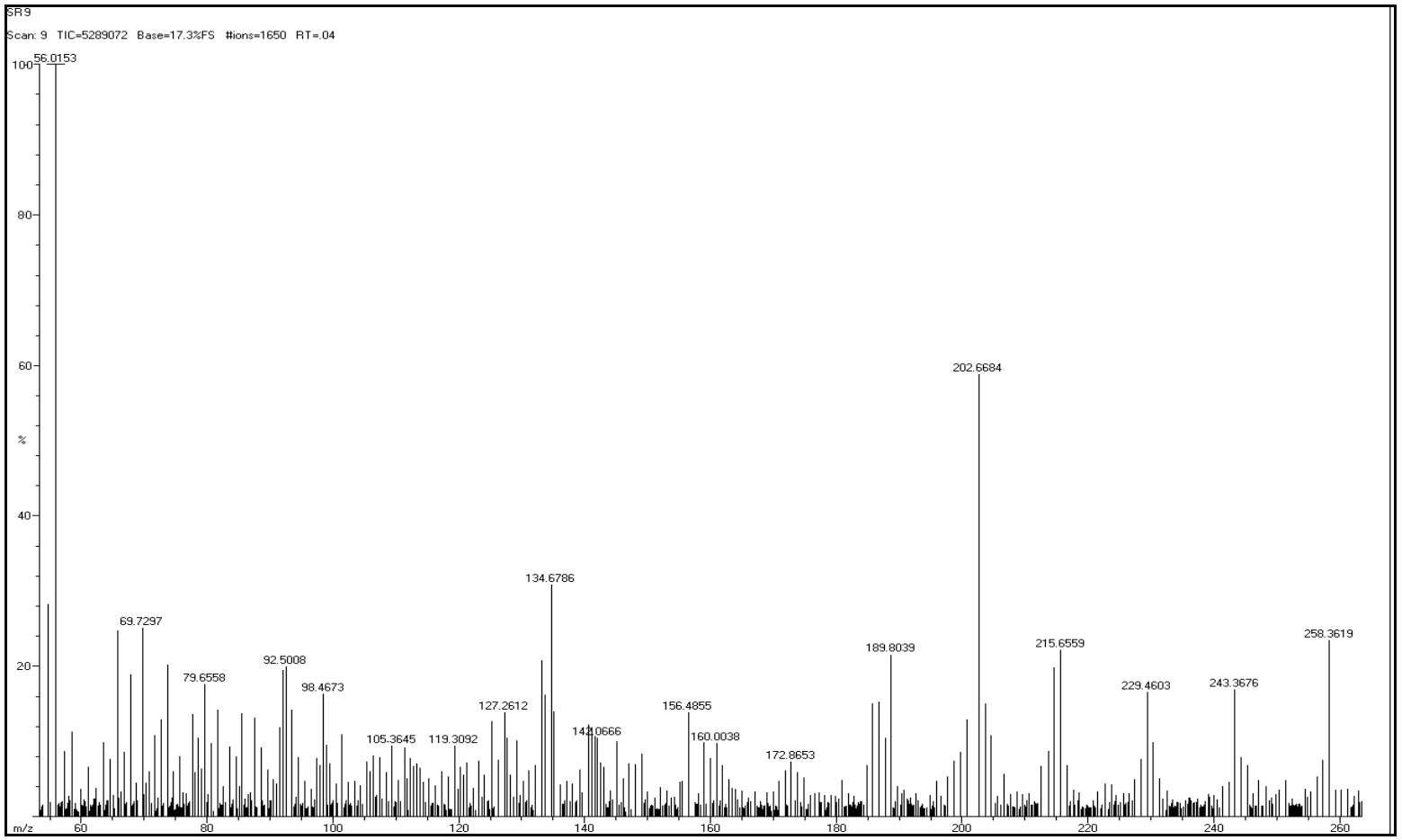


Fig 65: Mass spectrum of the compound SR_9

6.3. Table - 1: Physical and Analytical data of the synthesized compounds

Code No.	Name	Nature	Solubility	Molecular weight (g)	Molecular formula	Melting point (° C)	Percentage yield (% w/w)	R_f values (CH_3OH: H_2O) 8:2
SR_1	2-methyl-1-H-benzimidazole	Light yellow crystalline, solid	Freely soluble: Methanol. Soluble: Ethanol, benzene, acetone and chloroform. Insoluble: Water, hexane and ethyl acetate.	132.16	$C_8H_8N_2$	176 * (174-178)	60.73	0.92
SR_2	2-ethyl-1-H-Benzimidazole	Yellowish white crystals	Freely soluble: Methanol and acetone. Soluble: Benzene, ethanol, ethyl acetate and chloroform. Insoluble: Hexane and water.	146.18	$C_9H_{10}N_2$	211 * (213-215)	38.89	0.72
SR_3	2-propyl-1-H-benzimidazole	Light yellow crystals	Freely soluble: Methanol. Soluble: Benzene, ethanol, ethyl acetate, chloroform and acetone. Insoluble: Hexane and water.	160.21	$C_{10}H_{12}N_2$	183 * (180-184)	85.86	0.78

SR_4	1-((sulphanilamido) methyl)-2-methyl-benzimidazole	Blackish red crystals	Soluble: Methanol. Slightly soluble: Acetone, benzene, ethanol, ethyl acetate and chloroform. Insoluble: Hexane and water.	316.37	$C_{15}H_{16}N_4O_2S$	142	45.58	0.93
SR_5	1-((sulphanilamido) methyl)-2-ethyl-benzimidazole	Light yellowish white powder	Soluble: Acetone. Slightly soluble: Methanol, ethyl acetate and chloroform. Insoluble: Ethanol, benzene, hexane and water.	330.40	$C_{16}H_{18}N_4O_2S$	123	31.61	0.81
SR_6	1-((sulphanilamido) methyl)-2-propyl-benzimidazole	Black crystals	Soluble: Methanol, ethanol and acetone. Slightly soluble: Benzene, ethyl acetate and chloroform. Insoluble: Hexane and water.	344.43	$C_{17}H_{20}N_4O_2S$	149	28.45	0.82

SR_7	1-((piperazino) methyl)-2-methyl-benzimidazole	Light brown crystals	Soluble: Methanol. Slightly soluble: Ethanol, hexane, acetone, ethyl acetate and chloroform. Insoluble: Benzene and water.	230.30	$C_{13}H_{18}N_4$	148	71.17	0.86
SR_8	1-((piperazino) methyl)-2-ethyl –benzimidazole	Brownish crystals	Soluble: Methanol and ethanol. Slightly soluble: Chloroform, ethyl acetate, acetone and hexane. Insoluble: Benzene and water.	244.33	$C_{14}H_{20}N_4$	161	92.70	0.84
SR_9	1-((piperazino) methyl)-2-propyl-benzimidazole	Brownish crystals	Soluble: Methanol, ethanol and chloroform. Slightly soluble: Acetone, ethyl acetate and benzene. Insoluble: Hexane and water.	258.36	$C_{15}H_{22}N_4$	174	69.10	0.87

* Reported melting points. (Jubie S., *et al*., 2010; Kalirajan R., *et al*., 2009)

6. 4. A. Screening of antibacterial activity:

The synthesized compounds were evaluated *in vitro* anti bacterial activity against gram negative bacteria *Escherichia coli* MTCC 1302, *Pseudomonas aeruginosa* MTCC 741 and gram positive bacteria *Staphylococcus aureus* MTCC 740 and *Bacillus subtilis* MTCC 121. These are the agents commonly causes gastrointestinal tract infection and biliary tract infection. The gram negative organism *Escherichia coli* and *Pseudomonas aeruginosa* causes septicemia, gastroenteritis with mild to severe bloody diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome. The gram positive organism *Staphylococcus aureus* and *Bacillus subtilis* causes diarrhoea, pyogenic infection and septicemia.

As per the data obtained, it was confirmed that all the tested compounds possess antibacterial activity against both gram positive and gram negative organisms.

The compound SR_4 showed the significant activity in the following order: *Staphylococcus aureus* > *Pseudomonas aeruginosa* > *Bacillus subtilis* > *Escherichia coli*.

The compound SR_5 showed the significant activity in the following order: *Staphylococcus aureus* > *Pseudomonas aeruginosa* > *Escherichia coli* > *Bacillus subtilis*.

The compound SR_6 showed the significant activity in the following order: *Bacillus subtilis* > *Pseudomonas aeruginosa* > *Escherichia coli* > *Staphylococcus aureus*.

The compound SR_7 showed the significant activity in the following order: *Escherichia coli* > *Pseudomonas aeruginosa* > *Bacillus subtilis* > *Staphylococcus aureus*.

The compound SR_8 showed the significant activity in the following order: *Escherichia coli* > *Pseudomonas aeruginosa* > *Bacillus subtilis* > *Staphylococcus aureus*.

The compound SR_9 showed the significant activity in the following order: *Escherichia coli* ≥ *Pseudomonas aeruginosa* > *Bacillus subtilis* > *Staphylococcus aureus*.

However the antibacterial activity of all the tested compounds against the tested organisms was found to be less than that of standard antibacterial drug ciprofloxacin at tested dose level.

Table 2: *In vitro* antibacterial activity of synthesized compounds by disc diffusion method

Microorganisms	Diameter of zone of inhibition (in mm)												
	SR_4 (µg/ disc)		SR_5 (µg/ disc)		SR_6 (µg/ disc)		SR_7 (µg/ disc)		SR_8 (µg/ disc)		SR_9 (µg/ disc)		Ciprofloxacin (µg/disc)
	25	100	25	100	25	100	25	100	25	100	25	100	100
Staphylococcus aureus	17	21	17	25	12	17.3	12	15	11	15.6	10	16	28
Bacillus subtilis	13	17	11	14	21	27	11	16.4	12.6	17.5	13	16	29
Escherichia coli	12	17	13	17	19	22.3	20	25	21	27	14	17	30
Pseudomonas aeruginosa	15	18	17	22	20	24	17	21.5	21	26	14	17	32

6. 4. B. Screening of antifungal activity:

The synthesized compounds were evaluated for *in vitro* antifungal activity against two fungal organisms *Candida albicans* ATCC 24433 and *Trichophyton rubrum* ATCC 2327. These organisms cause serious dental infections. As per the data obtained, it was confirmed that all the tested compounds possessed anti-fungal activity. However, SR_9, **1-((piperazino) methyl)-2-propyl-benzimidazole** exhibited more potent anti fungal activity against both fungal organisms among the test compounds.

The compound SR_4 showed the significant activity in the following order: *Candida albicans* > *Trichophyton rubrum*. The compound SR_5 showed the significant activity in the following order: *Candida albicans* > *Trichophyton rubrum*. The compound SR_6 showed the significant activity in the following order: *Trichophyton rubrum* > *Candida albicans*. The compound SR_7 showed the significant activity in the following order: *Trichophyton rubrum* > *Candida albicans*. The compound SR_8 showed the significant activity in the following order: *Candida albicans* > *Trichophyton rubrum*. The compound SR_9 showed the significant activity in the following order: *Trichophyton rubrum* > *Candida albicans.*

However, the anti-fungal activity of the compound SR_9 against the tested organism was found to be less than that of antifungal drug ketoconazole at tested dose level.

Table 3: *In vitro* antifungal activity of synthesized compounds by disc diffusion method

Microorganisms	Diameter of zone of inhibition (in mm)												
	SR_4 (µg/ disc)		SR_5 (µg/ disc)		SR_6 (µg/ disc)		SR_7 (µg/ disc)		SR_8 (µg/ disc)		SR_9 (µg/ disc)		Ketoconazole (µg/ disc)
	25	100	25	100	25	100	25	100	25	100	25	100	100
Candida albicans	16	21	17	21	14	17	11	14	20	22	23	26	31
Trichophyton rubrum	12	15	14	17.5	16	19.1	13	18	17	20	24	29	30

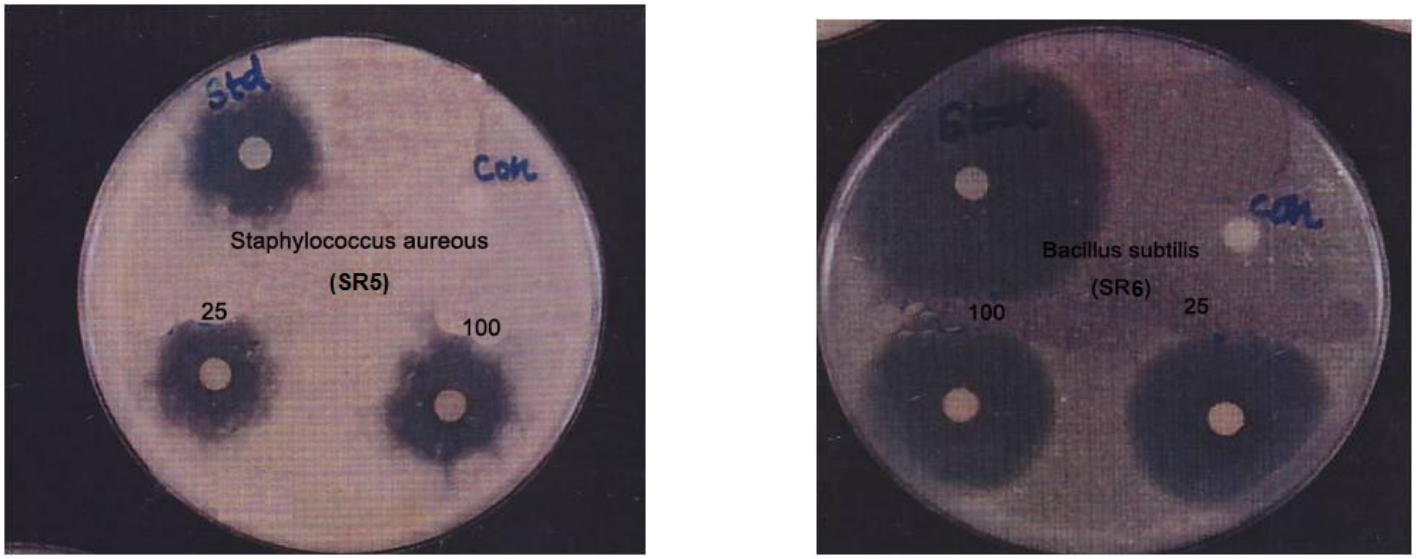


Fig 66: Antibacterial activity of tested compounds against tested microorganisms

Staphylococcus aureus* and *Bacillus subtilis

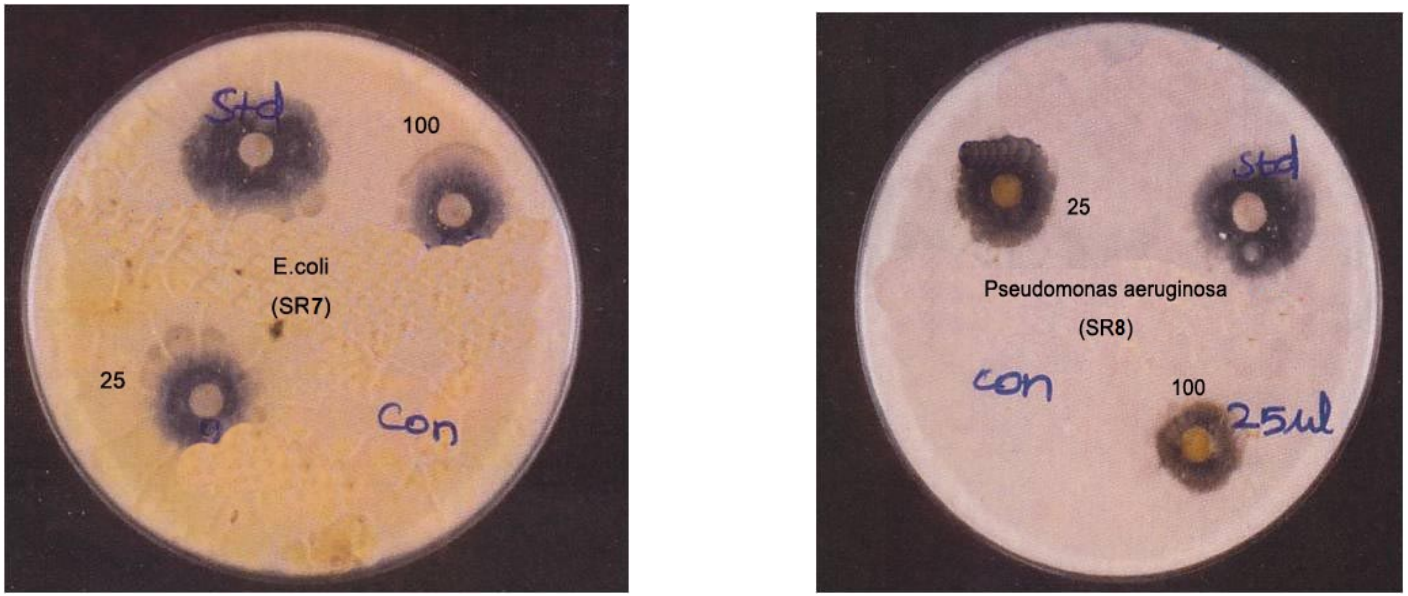
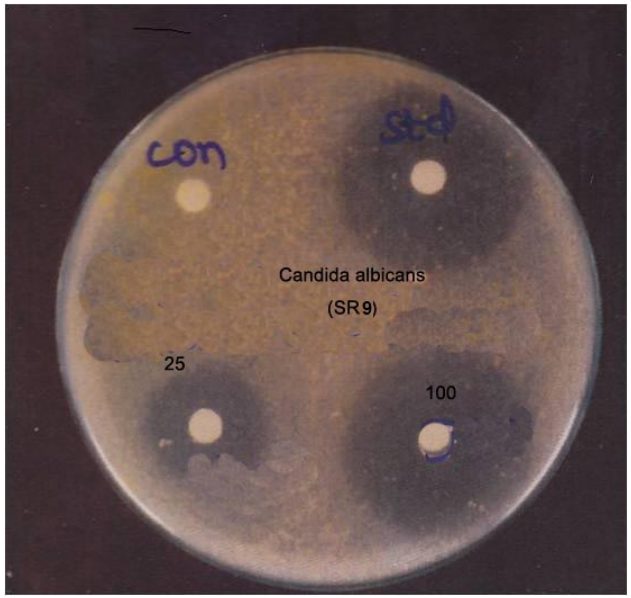


Fig 67: Antibacterial activity of tested compounds against tested microorganisms

Escherichia coli* and *Pseudomonas aeruginosa



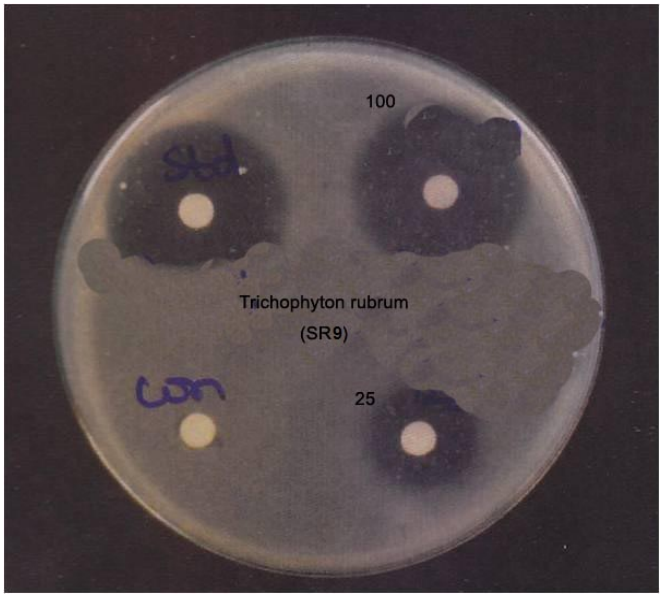


Fig 68: Antifungal activity of tested compounds against tested microorganisms

Candida albicans* and *Trichophyton rubrum

SUMMARY AND CONCLUSION

7. SUMMARY AND CONCLUSION

The current interest in the development of new antimicrobial chemotherapy has been the mainstay of medicinal intervention against infectious diseases caused by various pathogens. A matter of concern in the treatment of microbial infections is the limited number of efficacious antimicrobial drugs. There is a real perceived need for the discovery of new compounds that are endowed with antimicrobial activities, possibly acting through mechanism of action, to which many clinically relevant pathogens are now resistant (Uday Kalidhar., 2011). In order to expand the group of benzimidazole derivatives, we synthesized several new benzimidazole ring containing N-mannich bases. It has been observed that the presence of two (or) more heterocyclic moieties fused or linked enhance the biological profile of drug molecules by many folds.

The appropriate carboxylic acids were reacted with O-phenylene diamine to give the corresponding 2-substituted benzimidazole in good to excellent yields by Phillip's reaction. Then, a series of six novel mannich bases of 2-alkyl substituted benzimidazole derivatives were synthesized using mannich reaction by reaction with amines (primary and secondary) and formaldehyde. The purity of the synthesized compounds was checked by performing Thin Layer Chromatography (TLC) and determining melting points.

We reported our results from a study of replacing the N-1 hydrogen of novel benzimidazole derivatives with different types of substitutions like sulphanilamide and piperazine to form N-methyl substituted benzimidazole derivatives by mannich reaction. The structure of the synthesized compounds were elucidated by physical and spectral (UV, IR, ^{1}H NMR and Mass) analysis. The NH band (3164-3385 cm^{-1}) and NH proton signal (δ 4.80 – 5.0 ppm) of 2-substituted benzimidazole in IR and

^{1}H NMR spectrum respectively in the synthesized compounds (SR_1–SR_3), confirmed the formation of benzimidazole nucleus. In SR_1, ^{1}H NMR spectrum showed a singlet for 3 protons at δ 2.42 confirmed the substitution of methyl group at C_2 of benzimidazole nucleus. In SR_2, gave quartet peak for 2 protons at δ 2.59 and a triplet peak for 3 protons at δ 1.27 indicated the presence of ethyl group at C_2 of benzimidazole. In SR_3, a two triplet peak for 5 protons at δ 2.55 and δ 0.96 and a multiplet peak for 2 protons at δ 1.66 indicated the presence of propyl group at C_2 of benzimidazole.

The IR spectrum of each N-mannich bases of SR_4 to SR_6 showed the characteristic IR absorption bands in the region of 3376-3385 cm^{-1}, 3263-3289 cm^{-1} and 1312-1320 cm^{-1} due to the presence of primary amino, secondary amino and SO_2 stretching of sulphonamide moiety. The structural confirmation of each N-mannich bases of SR_4 to SR_6 was further made using ^{1}H NMR spectra. It showed signals at δ, ppm: 6.06 - 6.18 (2H, s, -CH_2 proton), 4.57 – 4.60 (2H, s, SO_2NH_2 proton) and 5.76 – 5.83 (1H, s, NH of sulphonamide). Thus, confirmed the proposed structures for above N-mannich bases of corresponding 2-substituted benzimidazole derivatives.

The IR spectrum of each N-mannich bases of SR_7 to SR_9 showed the characteristic IR absorption bands in the region of 3385 - 3416 cm^{-1} due to the presence of 2° N-H (secondary amino) streching of piperazine ring. The structural confirmation of each N-mannich bases of SR_7 to SR_9 was further made using ^{1}H NMR spectra. It showed signals at δ, ppm: 2.37 – 2.93 (8H, m, -CH_2 of piperazine), 5.47 – 5.61 (2H, s, CH_2 proton) and 1.13 – 1.15 (1H, s, NH of piperazine). Thus, confirmed the proposed structures for above N-mannich bases of corresponding 2-substituted benzimidazole derivatives.

The structural confirmation of synthesized compounds of SR_1 to SR_9 was further made using Mass spectra. The molecular ion (M^+) peaks such as 132.12, 146.21, 160.11, 316.37, 330.40, 344.43, 230.30, 244.33 and 258.36 for SR_1, SR_2, SR_3, SR_4, SR_5, SR_6, SR_7, SR_8 and SR_9 respectively corresponded with their molecular weights. The predicted chemical structure of title compounds was further supported by the fragmentation peaks.

The compounds were screened for their antibacterial and antifungal activities. The activities reported by means of zone of inhibition in millimeter. All the compounds showed very good antibacterial and antifungal activities at the tested dose level.

The sulphanilamide group containing N-mannich bases were more superior for inhibiting the growth of *Staphylococcus aureus* and *Bacillus subtilis* than piperazine containing N-mannich bases. Among the compounds, SR_4 to SR_6, the compound SR_5 was more active than the other compounds against the growth of *Staphylococcus aureus*. Likewise, the compound SR_6 was more active than SR_4 and SR_5 to inhibit the growth of *Bacillus subtilis*.

The piperazine group containing N-mannich bases were more superior for inhibiting the growth of *Escherichia coli* and *Pseudomonas aeruginosa* than sulphanilamide group containing N-mannich bases. Among the compounds SR_7 to SR_9, the compound SR_8 was more active than the other compounds against the growth of *Escherichia coli* and *Pseudomonas aeruginosa*.

Among the tested compounds, piperazine derivatives were more superior to sulphanilamide derivatives against gram negative bacteria. But sulphanilamide derivatives were more active than piperazine derivatives against gram positive bacteria.

The anti-fungal evaluation of compounds (SR_4 to SR_9), the piperazine group containing N-mannich bases were more superior for inhibiting the growth of *Candida albicans* and *Trichophyton rubrum* than sulphanilamide group containing N-mannich bases. Among the compounds of SR_7 to SR_9, the compound SR_9 was more active than the other compounds against the growth of *Candida albicans* and *Trichophyton rubrum*.

Even though, the anti microbial activity of tested compounds was less than their standard compounds are ciprofloxacin (antibacterial) and ketoconazole (antifungal) in the present study. In future study, it could be increased (or) equalized by altering the number of carbon atoms in side chain (or) introducing aromatic ring (or) substituted aromatic ring (or) heterocyclic ring (or) by introducing double bond in side chain in the 2^{nd} position of benzimidazole nucleus. In other way, the synthesis of 2-substituted benzimidazoles can be altering the complex with amines, like as pyrrolidine, imidazole, piperidine, morpholine and N-methyl piperazine etc. in the 1^{st} position of benzimidazole nucleus.

Since a fewer species have been used in this study, it was warranted to screen these compounds with varied species and resistant strains. Further experiments were needed to elucidate their exact mechanism of activity. These results suggest that the benzimidazole ring is an important pharmacophore in modern drug discovery and the tested derivatives of benzimidazoles have excellent scope for further development as commercial antimicrobial agents in the chemotherapeutic approach in human. Our findings will prove useful to those chemists, pharmacists, medicinal chemists who are interested in the synthesis of potential Mannich bases as drugs with minimum side effects and also have comparatively low cost.

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8. BIBLIOGRAPHY

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